THE INHERITANCE OF CERTAIN CHLOROPHYLL CHARACTERS IN MAIZE[1]

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INTRODUCTION

Many chlorophyll characters of maize have been discovered and their genetic behavior worked out. Some of these manifest themselves in the mature plant and others in the seedling. Some phenotypes are striped and others are totally devoid of chlorophyll. Also, there is one type of chlorophyll deficiency, the virescent seedling, in which the plants are all white (or yellow) with the exception of a little chlorophyll at the tips of the leaves. Virescent plants gradually turn green and in some cases reach maturity. The striped chlorophyll characters at maturity generally develop longitudinal stripes devoid of chlorophyll.

Some chlorophyll-deficient characters which differ markedly from any previously described and which are, more or less, the reverse of the virescent type mentioned above, are reported in this article. The description and method of inheritance of these are to be found in the following pages.

DESCRIPTION OF CHARACTERS

Maize plants possessing these characters start out in life with the presence of green chlorophyll, but the chlorophyll-deficiencies show

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themselves to some extent in the seedling stage. They may be described as follows:

1. *Yellow-white.* This is a seedling character only, as the seedling dies about the time the endosperm of the seed is used up. In initial stages the

FIGURE 1.—White-base-leaf plant with white stem and dark-green leaves.

seedlings are more or less green, but they turn yellow and white very quickly. In fact, they seldom ever have a full-green color. As the green fades they take on a yellow color so that a portion of the plant may be part white and part a greenish-yellow color, and finally, in some cases, entirely

white. The seedlings may die at any of these stages. Two genetic factors are responsible for this character, but the part played by either of the factors separately, when both are included in the same progeny, has not been ascertained.

In the later stages of their life these types resemble the yellows and whites reported by LINDSTROM (1918, 1921), but they differ in the earlier stages, in that they show streaks of green color.

FIGURE 2.—White-base-leaf plant with white stem and striped leaves.

2. *White-base leaf.* This character is apparent in some cases even in the seedling stage, but is very distinct when the plant is about half mature. Near maturity the white color at the base of the leaf blade gradually spreads outward into the leaf giving the leaf a mottled or striped appear-

ance. In some cases the stripes are very wide, even wider than in "*japonica*" (LINDSTROM 1918) and in some cases the stripes are finer than "fine-striped" (LINDSTROM 1918). Also, some plants show a mottled appearance, the leaves being extremely pale green or white with irregular areas of darker-green color. The upper leaves of some of these plants turn pure white when nearing maturity. As far as I have been able to observe, all of these variations are manifestations of the same genotype. Pictures of the white-base-leaf character are shown in figures 1 and 2.

The leaf sheaths in this type of chlorophyll deficiency are nearly white, resembling KEMPTON'S (1921) white-sheath plants. I have grown white-

FIGURE 3.—Maize seedlings showing zebra markings.

sheath plants alongside these white-base-leaf plants and found that, while white sheaths develop near maturity in the former, the white-base-leaf character shows in the latter at any time from the seedling stage to the time the plant is half mature. In KEMPTON'S "white-sheaths" the white areas start low on the plant and spread upward, while in the type here reported they make their appearance at the base of the leaves and gradually spread both ways, that is, out into the leaf blades and down the sheaths. Owing

to difference in time of maturity of the two types I have not yet succeeded in making crosses between them.

3. *Seedling zebra.* The markings in this type of chlorophyll deficiency resemble very closely those described in the mature-zebra type (DEMEREC 1921). The leaves show alternate bands of green and yellow, although in some cases the bands are more or less irregular (see figure 3). This type may either turn green or fade out into the yellow-white character or into the white-base-leaf character. In other words, the seedling-zebra character disappears soon after the plants have passed the seedling stage.

SYMBOLS

m_1 and m_2, factors responsible for the yellow-white character; either one of these factors or both produce the same effect.
w_l, a factor responsible for the white-base-leaf character.
z_{b2}, a factor for the seedling-zebra character.

MATERIAL

The original material upon which these studies were made, consisted of two ears sent in 1920 to Doctor E. W. LINDSTROM, then of the UNIVERSITY OF WISCONSIN, by Doctor E. G. ANDERSON, then of CORNELL UNIVERSITY. This material was turned over to the writer who was a graduate student at the UNIVERSITY OF WISCONSIN at that time. In what follows, these two original ears will be referred to as A 1222(3) and A 1315 (8).

The writer wishes to acknowledge his gratitude to Doctor LINDSTROM and Doctor L. J. COLE for their encouragement and helpful suggestions during the early part of these studies, and to Doctor J. L. LUSH for criticisms and suggestions.

The material was grown in 1920, 1921 and 1922, at the WISCONSIN AGRICULTURAL EXPERIMENT STATION, and in 1923 at the TEXAS AGRICULTURAL EXPERIMENT STATION.

THE BEHAVIOR OF THE YELLOW-WHITE CHARACTER

Self-fertilized ear A 1315 (8) (from CASTLE'S No. 123), which was heterozygous for some chlorophyll deficiencies, was the parent of all the progenies used in the studies of the yellow-white character. Self-pollination of plants from this ear failed to give good results and only three self-fertilized ears were obtained. These three ears segregated for the yellow-white character, in the greenhouse, the following winter. The counts are found in table 1.

TABLE 1

Segregation of green and yellow-white in progenies derived by self-fertilization from the original ear, A1315 (8) (from CASTLE'S *No. 123).*

PROGENY NUMBER	GREEN	YELLOW-WHITE
49 (2)	44	22
(10)	40	12
(11)	67	27

Another ear, A1222 (3) × A1315 (8), the product of a cross in which A1315 (8) was the male parent, furnishes additional information on the behavior of the yellow-white character. There was no segregation in the F_1 generation. The F_2 seedling counts from self-fertilized F_1 plants of this cross are shown in table 2. Five of the seven progenies deviate from a 3:1 ratio, less than three times the probable error, while the other two show a modified ratio which may be due to linkage of two recessive factors, either or both of which may produce the yellow-white condition. The factors m_1 and m_2 have been tentatively adopted to express this relation. Coupling of these two factors with 30 to 40 percent of crossing over would explain the two progenies with the modified ratios. It is to be noted that progenies 50(4) and 50(8) also deviate in the direction of an excess of yellow-whites. There are no progenies in table 2 which gave all green seedlings.

TABLE 2

Progenies in F_2 from the cross A1222(3) × A1315(8), showing both a 3:1 ratio and a modified linkage ratio.

PROGENY NUMBER	GREEN	YELLOW-WHITE	$\frac{\text{DEVIATION}}{\text{PROBABLE ERROR}}$ FOR 3 : 1 RATIO
50 (3)	40	15	0.46
(4)	70	32	2.22
(8)	50	21	1.32
(10)	78	29	0.75
(14)	15	4	0.59
(19)	40	43	8.36
(21)	148	110	9.76

In table 3 are found counts on progenies from the self-pollinated ear 50(4). This ear gave in F_2 a modified ratio. The F_3 progenies of this ear show three modified ratios and four 3:1 ratios. These three modified ratios would be expected if m_1 and m_2 were in the same linkage group and fairly close together.

TABLE 3

Data on one F_3 progeny of the cross A1222(3)×A1315(8). The self-pollinated parent was a green plant 50(4) (table 2).

PROGENY NUMBER	GREEN	YELLOW-WHITE	DEVIATION / PROBABLE ERROR FOR 3 : 1 RATIO
333 (3)	1	3	(Poor germination)
(4)	37	11	0.50
(8)	14	5	0.20
(10)	56	39	5.36
(13)	77	32	1.56
(14)	7	12	5.51
(15)	62	16	1.55
(18)	0	2	(Poor germination)
(20)	13	13	4.36

Other evidence comes from a cross of 331(2)×333(6). This cross was made in F_2 families of the original ANDERSON cross. The F_1 did not segregate for the yellow-white character. The counts in F_2 are shown in table 4, and indicate that one of the factors which produce the yellow-white character is isolated here and behaves as a normal monohybrid.

TABLE 4

PROGENY NUMBER	GREEN	YELLOW-WHITE	DEVIATION / PROBABLE ERROR FOR 3 : 1 RATIO
878 (1)	All	0	....
(3)	80	24	0.67
(4)	All	0	....
(6)	28	14	1.85
(7)	69	18	1.38
Total observed	177	56	
Calculated	*175*	*58*	
Deviation	+2	−2	

Further evidence comes from cross 61(10)×49(4). The male parent 49(4) was one of the three original white-base-leaf plants which appeared from planting the original ear A1315(8). The F_1 plants from this cross were all green. In F_2 the counts shown in table 5 were made.

Now, 388(14) (sister plant of the parents of the progenies just mentioned) was crossed by 345(12). This latter individual traces back to the original cross, A1222(3)×A1315(8). Both individuals, 388(14) and

TABLE 5

PROGENY NUMBER	GREEN	YELLOW-WHITE
388 (1)	80	36
(5)	All	
(13)	77	22
Total observed	157	58
Calculated, 3 : 1	*161*	*54*
Deviation	−4	+4

345(12), had the possibility of being heterozygous for the yellow-white character. In F_1 yellow-whites were not found. The counts in F_2 are given in table 6.

TABLE 6

PROGENY NUMBER	NON-YELLOW-WHITES	YELLOW-WHITES	DEVIATION / PROBABLE ERROR FOR 3 : 1 RATIO
893 (4)	107	43	1.54
(6)	41	24	3.29
(9)	13	2	1.55
(11)	All green		
(12)	55	13	1.66
Total observed for progenies (4) (9) (12)...	175	58	
Calculated, 3:1	*175*	*58*	
Deviation	0	0	

The counts on these progenies of family 893 were made in the winter of 1923-1924 under good greenhouse conditions. The types were very distinct and therefore subject to the least possible error. Three of the progenies were well within the limits of probability for a 3:1 distribution, while the other one barely exceeds the probable error.

THE INTERRELATION BETWEEN THE YELLOW-WHITE CHARACTER AND AN ALBINO

A cross 287(12)×329(5) yielded some data which indicate the possible interrelation of a factor for albinism and a factor for the yellow-white character. The yellow-white character seems to stand out clearly in these progenies as a greenish-yellow. The 329(5) individual which descended from 50(3) (table 2) has the possibility of being heterozygous for one of the

factors responsible for the yellow-white condition. The female parent (287(12)) of this cross, as shown by the progeny of a selfed ear, was heterozygous for one of the factors (w_2) responsible for albinism (STROMAN 1924). Factorially, the cross is $W_2\ w_2\ M_1\ M_1 \times W_2\ W_2\ M_1\ m_1$. The male parent (329(5)) was a white-base-leaf individual. The F_1 plants from this cross were all green. The F_2 distributions are shown in table 7.

TABLE 7

PROGENY NUMBER	GREEN	YELLOW-WHITE	WHITE	SEGREGATING RATIO
850 (2)	12	1	8	(?) (both)
(3)	26	0	11	3 : 1 (white)
(5)	20	0	3	3 : 1 (white)
(6)	14	6	0	3 : 1 (yellow-white)
(7)	20	6	7	9 : 3 : 4 (both)
(8)	17	5	0	3 : 1 (yellow-white)
Total observed for progenies (3) and (5)	46	0	14	Green : albino only
Calculated, 3 : 1	*45*	*0*	*15*	
Deviation	+1		−1	
Total observed for progenies (6) and (8)	31	11	0	Green : yellow-white only
Calculated, 3 : 1	*32*	*10*	*0*	
Deviation	−1	+1		
Total observed for progenies (2) and (7)	32	7	15	Green : yellow-white : albino
Calculated, 9 : 3 : 4	*30*	*10*	*14*	
Deviation	+2	−3	+1	

The observed distributions in all three cases fit their respective ratios. According to the assumed factorial composition of the parents, four kinds of F_1 progenies would be expected: namely, one pure for both dominant factors; one heterozygous for W_2 only; one heterozygous for M_1 only; and one which would be heterozygous for both factor pairs. The last three kinds of F_1 families were obtained, but no pure dominant appeared. Only eight F_1 plants were grown and six of these were selfed, giving 2 families consisting of 3 green : 1 albino; 2 families with 3 green : 1 yellow-white; and 2 families with 9 green : 3 yellow-white : 4 albino. The number of families is too small to make the absence of all-green families significant,

but at any rate it appears that the w_2 factor when homozygous inhibits all green, whether the M_1 factor is present or not.

Further evidence on the interrelation of a w factor and one of the factors for the yellow-white character comes from a cross, 852(3)×877(4). The 852(3) is an F_1 from a cross which might involve either w_1 or w_2 or both.

From self-pollinations of sister plants there seems to be only one w present and this w is apparently the w_2, since it seems to be linked to red- and non-red-stem seedlings (STROMAN 1924). The 877(4) traces to 50(21) which was heterozygous for both M_1 and M_2 factors. It has the possibility of being heterozygous for both the linked factors, M_1 and M_2; but there is no evidence to show whether it is, except by the results of the cross. Should it have been heterozygous for both M_1 and M_2, this cross was, factorially, $\widehat{M_1M_2}\ \widehat{M_1M_2}\ W_2w_2 \times \widehat{M_1M_2}\ \widehat{m_1m_2}\ W_2W_2$. Consequently, the F_1 might have contained eight different genotypes, which, when selfed, would give F_2 ratios as given in table 8.

TABLE 8

F_1 GENOTYPE	PHENOTYPIC F_2 SEGREGATION
2 $M_1M_1M_2M_2WW$	All green
2 $M_1M_1M_2M_2Ww$	3 green : 1 white
1 $M_1M_1M_2m_2WW$	3 green : 1 yellow-white
1 $M_1M_1M_2m_2Ww$	9 green : 3 yellow-white : 4 white
1 $M_1m_1M_2M_2WW$	3 green : 1 yellow-white
1 $M_1m_1M_2M_2Ww$	9 green : 3 yellow-white : 4 white
2 $M_1m_1M_2m_2WW$	9 green : 7 yellow-white, modified by linkage of m_1 and m_2
2 $M_1m_1M_2m_2Ww$	27 green : 21 yellow-white : 16 white, modified by linkage of m_1 and m_2

The F_2 counts are found in table 9 and the summaries in table 10. The test of genotypes is given in table 11.

The genotypic test is in nearly perfect agreement with the hypothesis, if the two progenies which are called modified 27 : 21 : 16 ratios are in reality the ones that were heterozygous for all three of the involved factor pairs. These two progenies under question do segregate for the two characters under discussion, namely, the yellow-whites and pure-white albinos. The results of this cross, then, further indicate that the w_1 (or w_2) factor, when homozygous, produce complete albinism, whether m_1 (or m_2) is present or not. The data on this cross serve also to further check the genetic behavior of the yellow-white character discussed in an earlier section of this paper.

TABLE 9

F_2 counts of the cross 852(3) $\widehat{M_1M_2}$ $\widehat{M_1M_2}$ Ww×877(4) $\widehat{M_1M_2}$ $\widehat{m_1m_2}$ WW. (F_1 all green).

PROGENY NUMBER	GREEN AND SLIGHTLY MOTTLED	YELLOW-WHITES	WHITES	APPROXIMATE RATIO
1064 (1)	15	0	4	3 : 1
(3)	68	0	16	3 : 1
(5)	28	10	11	9 : 3 : 4
(10)	41	14	0	3 : 1
(17)	61	13	0	3 : 1
(22)	10	0	1	3 : 1
(24)	84	12	61	Modified 27 : 21 : 16 (?)
(30a)	38	21	0	Modified 9 : 7
(30b)	9	10	0	Modified 9 : 7
(33)	15	0	0	Pure green
(34)	1	0	0	(Poor germination)
(42)	112	72	0	Modified 9 : 7
(44)	113	77	0	Modified 9 : 7
(45)	67	34	18	Modified 27 : 21 : 16 (?)
(47)	80	21	43	9 : 3 : 4

TABLE 10

Summary of F_2 counts which are shown in detail in table 9.

	GREEN TO MOTTLED	YELLOW-WHITE	WHITE	DEVIATION / PROBABLE ERROR
Observed of progenies 1064 (1), (3), (22)	93		21	
Calculated, 3 : 1	*86*		*28*	
Deviation	+7		−7	2.2
Observed of progenies 1064 (10), (17)	102	27		
Calculated, 3 : 1	*97*	*32*		
Deviation	+5	−5		1.5
Observed of progenies 1064 (30 a), (42), (44)	263	170		
Calculated, 22 : 14 ratio*	*265*	*168*		
Deviation	−2	+2		
Observed of progenies 1064 (5), (47)	108	31	54	
Calculated, 9 : 3 : 4	*109*	*36*	*48*	
Deviation	−1	−5	+6	P=0.498

* The 22 : 14 ratio is the modified 9 : 7 linkage ratio. This is figured by assuming 33 percent crossing over between M_1 and M_2, the two linked factors for the expression of the yellow-white character.

TABLE 11

CHARACTER OF SEGREGATION	NUMBER OF FAMILIES SHOWING EACH TYPE OF SEGREGATION		CALCULATED	o−c	(o−c)²	(o−c)²/c
	Expected ratio	Observed total				
All green	1	1	2.33	1.33	1.77	0.7596
3 green : 1 white	1	3	2.33	0.77	0.59	0.2532
3 green : 1 yellow-white	1	2	2.33	0.33	0.11	0.0472
9 green : 3 yellow-white : 4 white	1	2	2.33	0.33	0.11	0.0472
Modified 9 : 7	1	4	2.33	1.77	3.13	1.3433
Modified 27 : 21 : 16	1	2	2.33	0.33	0.11	0.0472
Total	6	14				2.4977

P=0.775

THE INHERITANCE OF THE WHITE-BASE-LEAF CHARACTER

One of the original self-pollinated ears, A1315(8), produced, on planting in the field, 9 full-green plants and 3 which were first called "new-striped." Later, plants of this "new-striping" were always seen to be associated with white color at the base of the leaves as the first indication of their mature-plant character; this character was then named "white-base-leaf."

One of these new white-base-leaf plants, 49(3), was used as the male parent in a cross with a full-green female, 59(3). The 59(3) parent was not related to 49(3) to any known extent. The F_1 plants from this cross, three of which were selfed, were all green. The resulting F_2 progenies consisted of 67 green and 21 white-base-leaf plants. This is obviously a 3:1 ratio due to the segregation of one factor pair. No F_3 progenies were grown from this cross.

In the F_1 of the cross, A1222(3)×A1315(8), the history of which has been discussed (p. 498), all plants were green. Five F_2 progenies were grown and in three of these segregation was found for the white-base-leaf character, giving 55 green and 20 white-base-leaf plants. This 3:1 ratio, together with the fact that two out of five progenies were pure green, tend to show that the segregation was due to a single factor pair. No F_3 progenies from this cross are available for analysis, but further evidence has been given by other crosses.

A full-green, 331(2), ×a white-base-leaf, 333(6), both parents of which trace back to the original cross, produced 6 full-green plants and 5 white-base-leaf plants. The 331(2) is thus shown to have been heterozygous for the white-base-leaf-producing factor, and as 333(6) was a white-base-leaf

plant, 1:1 ratios would be expected if the character in question was due to one recessive factor. Five of the green plants were self-pollinated and grown in the greenhouse the following winter; all segregated for white-base-leaf, i.e., mottled and white-base-leaf characters were noted in each progeny. The mottling is not sufficiently definite to allow the complete separation of the classes, due to the fact that in some of the white-base-leaf plants the mottling appears earlier than in others. Some plants show mottling in the seedling stage while others show it only in the mature stage. Early mottling may be due to a modifying factor, but as yet data have not been compiled to show that the mottling is genetically different from the white-base-leaf character. At any rate, all of the green plants from this back-cross were heterozygous for the white-base-leaf character, as expected.

A cross, 329(5)×345(9), in which both individuals were white-base-leaf plants tracing back to the same original cross, although belonging to separate F_2 families, gave only white-base-leaf plants when planted in the field.

Also, all F_3 progenies from the white-base-leaf plants of this cross, when selfed, consisted of white-base-leaf plants only. It is interesting to note, however, that some of these plants turned white, completely, when about half mature, while others turned white with the exception of a few lower leaves after they had reached maturity; some have, in addition to the white-base-leaf, a striping or mottling.

The evidence on the white-base-leaf situation is not complete. The data here presented show that one "main" factor is responsible for this character. Other data, not yet complete, leads me to believe that there is one modifying factor, possibly two.

INHERITANCE OF SEEDLING ZEBRA

Segregation with respect to seedling zebra seems to be due to the expression of one factor pair ($Z_{b2}z_{b2}$). Good counts were obtained from one family which was also characterized by the recessive factor for white-base-leaf w_l. The history of this family is as follows:

One selfed white-base-leaf individual (333(15)) in an F_2 progeny from the original cross grown in the field under the number 866, produced an F_3 family consisting of 12 green and 4 zebra. This was a seedling count made in the field. Seven self-pollinations were made in this family, five of which were heterozygous for the seedling-zebra character. The counts made in the greenhouse the following winter on the seedlings grown from the five heterozygous ears are given in table 12.

TABLE 12

PROGENY NUMBER	WHITE-BASE-LEAF	ZEBRA
866 (1)	9	7
(3)	86	34
(4)	55	17
(5)	79	31
(6)	17	2
Total observed	246	91
Calculated, 3 : 1	*253*	*84*

Deviation ÷ probable error = 1.3

From these counts, seedling zebra is evidently due to one recessive factor, but as these plants were all recessive for the w_l factor, it is not clear whether the latter factor has any effect on the expression of the seedling-zebra character. To settle this question it is necessary to have evidence from a family, some of which are not recessive for the w_l factor.

This requirement was met by family 878 which was the product of a cross between a full-green 331(2) and a white-base-leaf plant 333(6), both parents coming from the original cross A1222(3) × A1315(8). Five individuals of family 878 were self-fertilized, and all of the resulting progenies segregated for seedling zebra in the greenhouse. The counts are shown in table 13.

TABLE 13

PROGENY NUMBER	GREEN OR WHITE-BASE-LEAF	YELLOW-WHITE	ZEBRA
878 (1)	23	0	1
(3)	76	24	4
(4)	28	0	3
(6)	23	14	5
(7)	58	19	11
Total observed for progenies (1) and (4)	51	0	4
Calculated, 15 : 1	*52*	*0*	*3*
Deviations	−1	0	+1
Total observed for progenies (3) (6) (7)	157	57	20
Calculated, 45 : 15 : 4	*165*	*55*	*15*
Deviations	−8	+2	+5

P=0.3509

From these results seedling zebra appears to be dependent upon two recessive factors for its expression. The total counts in family 878 give 265 non-zebra plants and 24 zebra plants; this is fairly close to a 15:1 ratio, the calculated 15:1 for these progenies giving 270:18. In the families showing the presence of both z_{b2} and m_1 for seedling zebra and yellow-white characters, respectively, three factors seem to have been involved. The other factor necessary for production of seedling zebra in the presence of z_{b2} is probably the factor w_l, for white-base leaf. It has been shown that in family 866 (table 12), in which the white-base-leaf character was present and homozygous, a 3:1 ratio for zebra is obtained, the behavior being that of a simple monohybrid. The 45:15:4 ratio shown in table 13 represents a trihybrid segregation probably involving W_lw_l, $Z_{b2}z_{b2}$ and M_1m_1, in which the yellow-white character appears whenever m_1 is present unless both w_l and z_b are present; in the latter case the seedling will show the zebra marking even though the plant turns white on further development. Since, as previously mentioned, this was a back-cross with respect to the w_l factor, all the full-green plants must be heterozygous for the W_l factor. Why all were heterozygous for the Z_b factor is not known at this time.

CONCLUSIONS

These data and results are not offered as a final analysis of the whole situation with respect to the factors responsible for the white-base-leaf, yellow-white and seedling-zebra characters, but it is hoped that further study of these factors will bring to light any additional relationships which may exist.

These data indicate that two linked factors are responsible for the expression of the yellow-white character; one factor differentiates the white-base-leaf character from green (there is possibly a modifying factor for this character, but so far data are not sufficiently conclusive); and two factors are responsible for the seedling-zebra character, one of which is probably the w_l factor.

LINKAGE RELATIONS OF W_l AND S_u

The F_2 of the original cross, A1222(3)$\times$A1315(8), was segregating both for green and white-base-leaf plants and for starchy and sugary endosperm. The sugary factor (s_u) came from the female parent while the white-base-leaf factor came from the male parent. The repulsion phase of linkage should be found if these two factor pairs are linked. The data are given in table 14.

TABLE 14

PROGENY NUMBER	STARCHY SEED		SUGARY SEED	
	Green $S_u W_l$	White-base-leaf $S_u w_l$	Green $s_u W_l$	White-base-leaf $s_u w_l$
50 (2)	21	8	3	0
(3)	7	3	2	1
(4)	16	8	6	0
Total observed	44	19	11	1
Calculated, 25 percent crossing over	*39*	*18*	*18*	*1*
Deviations	+5	+1	−7	0

P=0.337

The assumption of 25 percent crossing over between S_u and w_l as well as between s_u and W_l, agrees fairly well with the data on these three F_2 progenies. These data are not conclusive as to the exact degree of linkage, but they do indicate that w_l and s_u are in the same linkage group.

LINKAGE RELATIONS OF THE FACTOR PAIRS, Yy AND M_1m_1

Two F_3 families from the original cross segregated for both yellow and white endosperm (Yy) and green and yellow-white leaf characters (M_1m_1). The Yy factor pair is presumably the same as that used by EMERSON (1921). These two families gave an approximation to a 3:1 ratio for each of these two factor pairs, as shown in table 15.

TABLE 15

PROGENY NUMBER	$Y\ M_1$	$Y\ m_1$	$y\ M_1$	$y\ m_1$
333 (13)	67	30	10	2
(15)	44	13	18	3
Total observed	111	43	28	5
Calculated, 33 percent crossing over	*99*	*42*	*42*	*5*
Deviations	+12	+1	−14	0

P=0.106

Further evidence came from the cross 331(2)×333(6) mentioned earlier in this paper. All progenies from this cross which segregated for the yellow-white character (M_1m_1) gave 3:1 ratios. Two progenies were found which segregated for both the y and M_1m_1 factor pairs as shown in table 16.

TABLE 16

PROGENY NUMBER	$Y\ M_1$	$Y\ m_1$	$y\ M_1$	$y\ m_1$
878 (6)	21	14	7	0
(7)	48	15	21	4
Total observed	69	29	28	4
Calculated, 33 percent crossing over	*68*	*29*	*29*	*4*
Deviations	+1	0	−1	0

Since the two factors m_1 and m_2 seem to be linked together with about 33 percent crossing over and one of these two factors is linked to the Y factor, it is desirable to offer the F_2 data from the original cross, A1222(3) ×A1315(8) as bearing on these relationships. These data are found in table 17.

TABLE 17

The F_2 generation of the cross A1222(3)×A1315(8) showing the relation of yellow and white endosperm to yellow-white seedlings.

PROGENY NUMBER	YELLOW SEED		WHITE SEED		RATIO OF GREEN TO YELLOW-WHITE
	Green*	Yellow-white	Green*	Yellow-white	
50 (3)	27	12	13	3	3 : 1
(4)	56	23	14	9	Modified 9 : 7
(8)	36	16	14	5	Modified 9 : 7
(10)	55	20	23	9	3 : 1
(14)	8	4	7	0	3 : 1
(21)	106	85	42	25	Modified 9 : 7
(19)	30	30	10	13	Modified 9 : 7

* The green classes include chlorophyll deficiencies other than the yellow-white condition.

Three of the progenies show a 3:1 ratio of green to yellow-white seedlings. It is doubtful whether one of these progenies (50(14)) shows linkage between the m and the Y factors. The other two 3:1 progenies seem to be independent of Yy, as is shown in summary in table 18.

From the above data it seems that one of the factors for the yellow-white seedling character is independent of the Y factor.

If M_1 and M_2 are linked, with 33 percent crossing over, Y and m_1 likewise linked, with 33 percent crossing over, and Y and m_2 completely linked, we should obtain, when segregation is taking place with respect to all three factors, a ratio of 22 yellow endosperm and green seedlings : 5 yellow endosperm and yellow-white seedlings : 0 white endosperm and

TABLE 18

Summary of the counts from table 17 of progenies 50(3) and 50(10).

	YELLOW SEED		WHITE SEED	
	Greens YM_2	Yellow-white Ym_2	Greens yM_2	Yellow-white ym_2
Observed	82	32	36	12
Calculated, 9 : 3 : 3 : 1	*91*	*30*	*30*	*10*
Deviations	−9	+2	+6	+2

P=0.46

green seedlings: 9 white endosperm and yellow-white seedlings. In the progenies in which only one factor for yellow-white seedlings was present, I found no case of complete linkage. There were very few progenies, however, which were segregating for both Y and M_1 (or M_2), and the failure to find a case of complete linkage does not prove that such linkages do not exist. In F_2 progenies of the original cross segregation occurred for all three factors (Y, M_1 and M_2), and this cross may, therefore, throw some light on their linkage relations. Counts of the F_2 progenies of the original cross, A1222(3)×A1315(8), are found in table 17 and shown in summary in table 19.

TABLE 19

Summary of progenies 50(4), (8), (21) and (19) (from table 17).

	YELLOW SEED		WHITE SEED	
	Green	Yellow-white	Green	Yellow-white
Observed	228	154	80	52
Calculated,* 251 : 181 : 92 : 52	*224*	*162*	*82*	*46*
Deviations	+4	−8	−2	+6

P=0.73308

* This is the ratio figured for a repulsion with 33 percent crossing over between Y and M_1 and 33 percent crossing over between M_1 and M_2 with Y and M_2 so loosely linked as to make detection of linkage impossible. Also, as has been explained, either M_1 or M_2, or both, cause the expression of the yellow-white condition.

This is a remarkably good fit, especially in comparison with that shown if independence of all three factors be assumed, the latter assumption

giving P equal to only 0.38. However, the peculiar gametic ratio required to fulfill the hypothesis of linkage and the assumed behavior of M_1 and M_2, is not very different from a non-linkage gametic ratio for these three factors. The evidence on these linkage relations, derived from the previously-mentioned progenies, led to the formulation of the hypothesis, and it is seen that the three abnormal progenies of table 17 do fit in and substantiate the hypothesis of 33 percent crossing over between Y and M_1 and 33 percent crossing over between M_1 and M_2, while Y and M_2 are 50 units or more apart, that is, in effect, independent. It must be admitted, however, that comparatively few progenies are available, from which to draw conclusions, and that the hypothesis remains correspondingly tentative.

SUMMARY

1. The expression of a seedling chlorophyll character, yellow-white, which is the reverse of virescent, is shown to be the result of m_1 or m_2, or both m_1 and m_2 factors. These two factors seem to be linked together with 30 to 40 percent crossing over.

2. A chlorophyll character named white-base-leaf is shown to be due to the presence of one recessive factor (w_l). The character may be noted any time from the seedling stage until the plant is fully mature. Its full expression appears when it is nearing maturity.

3. Seedling zebra, a seedling chlorophyll character, is expressed as a result of two recessive factors z_{l2} and w_l.

4. Where an m factor and an albino factor (w_2) are both heterozygous within the same progeny (MmW_2w_2), 9:3:4 ratios are obtained, showing that the w_2 factor inhibits all chlorophyll development even in the presence of m_1 (or m_2).

5. Data are presented which indicate that w_l occurs in the same linkage group as the factor for sugary endosperm (s_u).

6. Data are given, also, which seem to place m_1 (a factor for yellow-white seedlings) in the Y (factor for yellow endosperm) linkage group.

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BIONOMIC STUDIES ON CERTAIN TELEOSTS (POECILIINAE)
I. STATEMENT OF PROBLEMS, DESCRIPTION OF MATERIAL, AND GENERAL NOTES ON LIFE HISTORIES AND BREEDING BEHAVIOR UNDER LABORATORY CONDITIONS[1]

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INTRODUCTION

This paper constitutes the first report on investigations begun in 1918 on hereditary behavior in several genera of fishes belonging to the Poeciliinae, with special reference to the origin of specific differences in the group. It is primarily concerned with a discussion of the problems involved and with a description of the materials and methods of investigation used; together with some general notes on the breeding habits and behavior of the material under laboratory conditions.

It has, of course, been necessary to carry out genetic analyses of both genera before undertaking an analysis of the inter-generic hybrids, or any extensive special experiments under changed environmental conditions, in order to determine the hereditary behavior of these creatures under usual laboratory conditions. Furthermore, I have thought it desirable to make a fairly complete physiological survey of the material. The physiological studies nearing completion or upon which considerable data are now available include: General metabolic conditions of the material in relation to age and sex; susceptibility of the material to various environmental factors such as high and low temperatures and a number of chemical substances, including acclimation to these factors.

[1] The work which forms the basis of this paper was done in the Zoölogical Laboratory of the UNIVERSITY OF CHICAGO.

It is expected that these studies will furnish some basis for estimating the "adaptive" or survival value of a number of the special characteristics exhibited by these fishes. There are also a number of special problems pertaining to sex in these animals. Sex ratios are decidedly irregular and there is great variation in the age at which the fishes become sexually mature. Intersexes certainly occur, especially in certain of the generic hybrids.

The origin of specific differences, as a part of the problem, is mentioned here with some reluctance. While I like to regard it as the central theme of the investigation, its mention at this time is justified largely only by the desirability of indicating the trend of the work. There is much to be done in the way of genetic analyses both morphological and physiological before any intelligent analyses of this phase of the work can be made. Since the few data thus far obtained concerning the origin of specific differences are not in presentable form, discussion of this phase of the work may be postponed.

MATERIAL

Five genera are represented in my aquaria: *Xiphophorus helleri*, *Platypoecilus maculatus* (Günth.), *Lebestes reticulatus*, *Poecilia vivipara* and *Heterandria formosa*. The descriptions following apply especially to *X. helleri* and *P. maculatus*, with which most of the work thus far has been done. However, the habits and life histories are much the same for all of the species mentioned.

All of the material used thus far has been bred from specimens obtained from local fish fanciers with the exception of six male *X. helleri* that were obtained from a dealer in New Orleans. It was claimed by the dealer that these six males had been brought in directly from nature in Mexico. How long the material, other than the six males just mentioned, has been bred in captivity by the fish fanciers I have no way of knowing; but probably the fish came from material imported from Germany prior to 1914. Most of the aquarium fish used by fanciers in this country,—except endemic species,—I understand, have been imported by way of Germany.

The "purity" of the material is therefore open to some question. This is especially true of *X. helleri* and *P. maculatus*, two genera that can be hybridized without great difficulty. But since the two forms are so strikingly different in size, form and color, and since I am familiar both with the "pure" species and their hybrids, I do not feel that the genetic purity of the original material need be considered seriously. Moreover, since the two genera overlap in nature, material from that source must be open to

the same question and in any instance must be subjected to genetic analysis. It may be mentioned here that the "crescent-tail" (plate 1, figure 4) and the "orange-tail" (plate 1, figure 2) varieties of *X. helleri* are reputed (by fish fanciers) to be of hybrid origin.

All of these fishes are viviparous, fertilization being effected with the aid of the anal fin, which, in males becomes modified to form an intromittent or contact organ. In the act of copulation the male after displaying himself about the female for a few moments, approaches her from behind and a little below. With the modified anal fin extending laterally and the distal end directed somewhat anteriorly, the male makes a sudden dart forward and upward effecting a momentary contact of the organ with the genital opening of the female. The act may be repeated half a dozen times in a few seconds.

The gestation period varies somewhat in the different genera and even in females of the same species. It approximates four weeks, varying from about twenty days to six weeks or more. The average interval between broods for *X. helleri* is 39 days; for *P. maculatus*, 31 days. Nutrition within a temperature range of approximately $22 \pm 4°C$, appears to be the primary factor in determining the amount of this variation within the species. It must be said, however, that my information on this point is from general observation rather than specific experiment. There appears to be some positive correlation between the number of young in the last brood and the interval between it and the next brood; i.e., the interval seems to increase slightly with the number of young produced by the female.

The young are seven to ten millimeters long at birth and swim actively about. The yolk sac is much reduced at this time and is hardly noticeable. Anywhere from one or two to a hundred young may be born at one time, although twenty-five or thirty is a usual number. For example, in 100 broods taken at random from the records there were 2790 young. As many as ten broods have been obtained from a single female.

The rate of growth of young appears to be determined, within the usual temperature range, largely by food, although extremes of temperature play a part. They reach sexual maturity in anywhere from two months to a year (see page 524). One does not usually obtain more than two generations a year or perhaps five generations in two years.

LABORATORY METHODS

The fish are readily kept under laboratory conditions, and although the routine work is tedious no special difficulties are encountered. The

laboratory, a greenhouse room 30×40 feet, is kept at a temperature that varies one or two degrees from 24°C. During the mid-summer months the temperature in the tanks occasionally reaches 30° during the middle of the day. A large ventilating fan, keeps the air in the room in circulation at all seasons of the year and in addition during warm weather pulls the air through a large air washer which aids in keeping the temperature down. The air circulation is necessary for the maintenance of a uniform temperature over the entire room and especially prevents those tanks near the radiators becoming warmer than those elsewhere in the room.

Aquaria of various sizes are used. For matings, tanks 20×20×25.5 cm (10 liters), are used. The growing young are kept in tanks 25×25×31cm, (18 liters), or larger, depending upon the number and size of the fish. Although no hard-and-fast rule is followed closely we attempt to give about one liter of water to each gram of fish.[2] The water used in the tanks comes from a well under the laboratory. The city water supply is unsatisfactory on account of the chlorine added to it. The water in the tanks is changed no oftener than is necessary to keep the tanks clean and sweet. About two inches of well-washed gravel is placed in the tanks to furnish root-hold for such aquatic plants as *Valisneria spiralis*, *Ludwigia aldimiafolia*, and others.

The fish are fed on a prepared food which is a mixture of dried shrimp, fish roe, beef, cereal, egg yolk, dried algae, and a little magnesium sulphate. The materials are mixed into a stiff dough, baked hard and ground. Once a week or oftener they are fed some fresh or live food,—daphniae or other entomostraca, fresh fish roe, scraped frog legs, beef liver, beef steak, etc.

The environmental factors most easily regulated are, of course, temperature, food, and to some extent the chemical content of the water, and light. Temperature and food conditions have been mentioned. No attempt is made to regulate light conditions beyond avoiding direct exposure to sunlight during the summer months. During the winter months, however, I find it desirable to expose the tanks to sunlight. About April each year the glass roof and side walls are white-washed or painted. The roof and walls are then cleaned in the fall. Other environmental factors that must be taken into account include the accumulated wastes of the fish and other organisms in the water with them,—protozoa, snails, algae, other plants, etc. A tank with the proper number of plants and not too many fish together with a few snails for scavengers will main-

[2] The mature fish vary considerably in weight, but in general *P. maculatus* will average about 0.4 to 0.5 gram and *X. helleri* about 0.9 to 1.0 gram. The newly born fish weigh around 5 to 8 milligrams.

tain itself in good condition for several months without attention. A tank in which has been placed more food than the fish will consume gets stale very quickly. On the average I find it desirable to clean each tank thoroughly about once in four weeks.

TABLE 1

CHARACTERS	*Xiphophorus helleri*	*Platypoecilus maculatus*
Dorsal fin rays	11 to 16; M= 12.8±0.009	9 to 12; M= 10.0±0.004
Shape of dorsa lfin	Anterior margin slightly rounded; posterior margin sharply angular, —more marked in males	Strongly rounded. Similar in both sexes
Markings in dorsal fin	Scattered red spots	Usually none. When present, black
Anal fin,— males	Third produced ray ending in a retrorse hook and the anterior branch of the second segmented throughout. Some variation in detailed structure	Third produced ray without a hook and the anterior branch of the second ray not segmented distally. Variable in detailed structure
Pelvic fin	First ray about one-third length of second in males; about two-thirds length of second in females. Deep notch between 1st and 2nd in males	Similar to *X. helleri* (see text)
Caudal fin	In males 3rd to 14th ventral rays prolonged into a "sword" about length of body	Similar in both sexes. No sword
Form index $\left(\frac{\text{Body length}}{\text{Greatest depth}}\right)$	Males average 3.71±0.0161 Females average 3.17± 0.0065	Probably no significant difference between males and females
Color (see text)		
Age at which males mature	Variation 8 to 45 weeks Average 21.8 weeks	Variation 5 to 44 weeks. Average 14.7 weeks

Pedigree records are kept which include observations on the characters listed in the right-hand column of table 1, the age at which males mature,

and of course the sex ratios. Fish that die or are killed, are preserved in formaldehyde. Owing to the difficulty of making concise records of some of the color characters or patterns many of the specimens are photographed and the prints attached to the records of the specimens. A millimeter rule and a slip bearing the record number of the specimens are placed in the plane of the fish and photographed at the same time. One is thus able to make certain size measurements of the fish without having to take into account any enlargement or reduction in the photograph.

Thus far the only measurements recorded are of the body length and its greatest depth. The body length is taken as the distance from the tip of the snout to the end of the hypural. Since the position of the hypural must, in the great majority of cases, be estimated, some error is unavoidable. Likewise the greatest depth cannot be measured with great accuracy. The depth of the fish varies somewhat with the fullness of the digestive tract, and, in pregnant females, with the number and size of the young she is carrying. Measurements are taken with a caliper rule reading to tenths of a millimeter, although the measurements are usually recorded simply to the nearest millimeter.

TABLE 2

INDEX	MALES	FEMALES	INDEX	MALES	FEMALES
2.4	0	1	3.6	25	6
2.5	0	2	3.7	28	1
2.6	0	2	3.8	15	0
2.7	0	6	3.9	19	0
2.8	2	4	4.0	6	0
2.9	0	14	4.1	17	0
3.0	0	50	4.2	13	0
3.1	1	87	4.3	10	0
3.2	17	76	4.4	7	0
3.3	19	80	4.5	2	0
3.4	25	33	4.6	2	0
3.5	22	14	Total	230	317

Males, M=3.71±0.0161; S.D.=0.3611±0.0114
Females, M=3.17±0.0065; S.D.=0.1858±0.0046
Difference=0.54±0.0174

Since the form index,—greatest depth divided into body length,—is diagnostic to some extent of the species involved, and of maleness and femaleness in *X. helleri* at least, it is necessary to establish some basis for estimating the magnitude of the unavoidable errors of measurement. To furnish such a basis ten specimens of *X. helleri* were taken at random and

placed each in a separate container. An assistant brought the fish to the observer one at a time and in any order that pleased him. He also recorded the measurements as the observer called them. The observer never knew which fish was which, nor was any more—or any less—care used in making the measurements than in routine work. Each fish was measured ten times. The ten measurements of body length, greatest depth, and the calculated form index, were averaged for each of the ten fish. The amount by which each measurement deviated from the mean value for each set of ten measurements was expressed on a percentage basis. The greatest deviation in the 100 measurements was, for the form index, 6 percent, the least was 0. Each of these values occurred once. The mean percentage deviation was 1.67 ± 0.081 with a standard deviation of 1.206 ± 0.057. But it appears that for comparison of individual fish, each measured once, any difference less than six percent of the indices involved is of doubtful significance. Where averages of a considerable number of fishes are involved, differences that exceed two percent of the indices are probably significant.

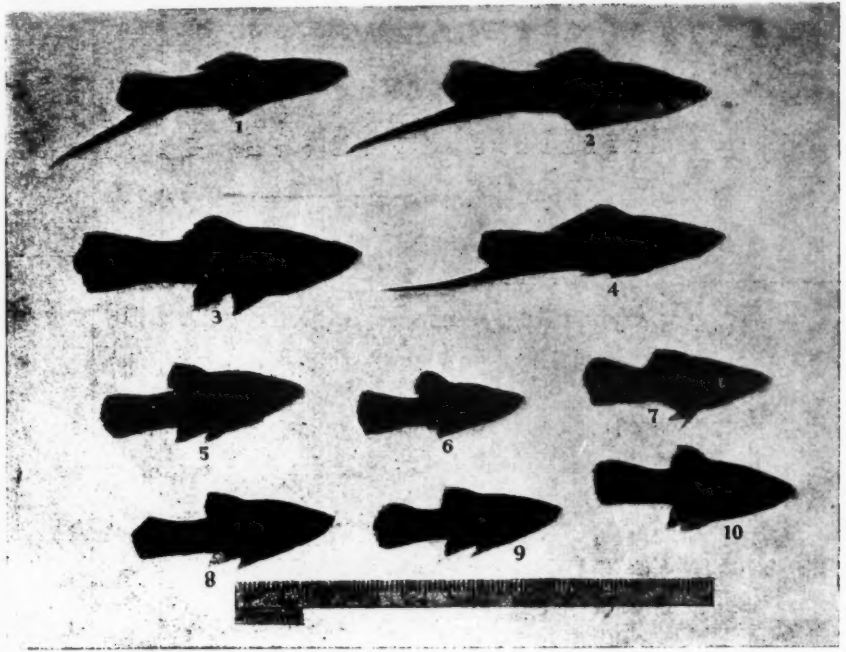
DIAGNOSTIC CHARACTERS OF *Xiphophorus helleri* AND *Platypoecilus maculatus*

A number of the diagnostic characters of *X. helleri* and *P. maculatus* are listed in parallel columns in table 1. The specimens are illustrated in plate 1.

In addition to the differences in number of rays and shape of the dorsal fin noted for the two genera in table 1 (see also table 3), the fin rays differ decidedly in thickness. They are relatively slender and of about the same diameter to the point of bifurcation in *X. helleri* and decidedly thick in

TABLE 3

NUMBER OF RAYS	FREQUENCY	
	P. maculatus	*X. helleri*
9	96	—
10	2082	—
11	102	12
12	1	618
13	—	1242
14	—	201
15	—	19
16	—	1
	M= 10.0±0.004	M= 12.8±0.009


1
2
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9
10

GENETICS 9: N 1924

LEGENDS FOR PLATE 1

The contrast between the several varieties is much greater in the living specimens than is indicated by the photographs. The ten fish were photographed at the same time under water, immediately after killing.

FIGURE 1.—*Xiphophorus helleri* male "green-tail"
FIGURE 2.—*X. helleri* male "orange-tail"
FIGURE 3.—*X. helleri* female "crescent"
FIGURE 4.—*X. helleri* male "green-tail crescent"
FIGURE 5.—*Platypoecilus maculatus* female "W"
FIGURE 6.—*P. maculatus* male "W" with spot
FIGURE 7.—*P. maculatus* male "W" with crescent
FIGURE 8.—*P. maculatus* female "P"
FIGURE 9.—*P. maculatus* female "N"
FIGURE 10.—*P. maculatus* female "R"

P. maculatus, tapering abruptly before bifurcating. There is great variation in the number and extent of the distal bifurcations of the dorsal fin rays in both genera.

TABLE 4

Inheritance of dorsal-ray number in X. helleri

NUMBER OF DORSAL RAYS OF PARENTS	NUMBERS AND FREQUENCIES OF DORSAL RAYS IN OFFSPRING					
	11	12	13	14	15	MEAN
Both parents 12 dorsal rays	3	238	229	6	0	12.50±0.0112
Both parents 13 dorsal rays	8	128	239	26	0	12.71±0.0247
Both parents 14 dorsal rays	0	28	194	47	0	13.05±0.0356
Both parents 15 dorsal rays	0	0	1	29	13	14.28±0.0512

Color patterns

The color pattern of *X. helleri* is subject to relatively little variation. The variations that do occur affect the shade of red in the longitudinal stripe,—reddish orange to deep red; the occasional presence of secondary lateral red stripes above or below or both above and below the main stripe. Occasionally one sees one or more black vertical bands of pigment on the side of the fish just back of the pectoral region. Whether or not these are heritable differences has not yet been determined. However, their much accentuated appearance in many of the generic hybrids indicates that they are heritable characters. One strain is characterized by the presence of a crescent of black pigment at the base of the caudal fin. This appears to be a simple Mendelian character dominant to the unpigmented condition of the base of the caudal fin. Another strain is characterized by an orange-colored stripe on the "sword." The hereditary behavior of this character is more difficult to analyze than that of the "crescent" variety, since the sword tail upon which the orange color appears is strictly sex-limited. It can, however, be inherited through the female, although we are thus far unable to distinguish females carrying the factor (or factors) for this character from those that do not. The orange stripe is dominant to the usual greenish stripe. In addition to being sex-limited it also may be sex-linked. The details of the hereditary behavior of these characters will appear in a subsequent report.

The color patterns of the several varieties of *P. maculatus* are highly variable. Four varieties are represented in my aquaria: White (W), an unmarked fish or with the posterior margins of the scales faintly edged

with small roundish melanophores (plate 1, figure 5); Nigra (N), figure 9, is marked by a broad black band of closely grouped large melanophores extending usually from the pectoral region to the base of the caudal fin. The band often extends to the eye and varies a good deal in intensity. Pulchra (P), figure 8, is marked in a salt-and-pepper fashion by scattered melanophores, that here and there are grouped in bunches. The ground color is white or faintly olivaceous with no traces of red. Rubra (R), figure 10, is much like P except that the fish is of a variable red color—varying from a pale reddish straw color to a bright red. The deeper reds are seen only in males and then only when mature. The young show no trace of red. Further, the scattered groups of melanophores are usually less abundant and may in extreme cases be almost completely absent. The color of the Rubra appears to be due to the presence of relatively small uniformly distributed erythrophores.[3]

Although these races of *P. maculatus* are rather variable there is never the slightest difficulty in distinguishing one from another. However, one cannot always be sure of separating PR from PP or RR until the fish are well past maturity. Some of the other hybrids between these races present the same difficulty.

The factors for W, N, P and R constitute a sex-linked multiple allelomorphic series, as was indicated in the abstracts of papers presented at the 1922 Boston meetings of the AMERICAN SOCIETY OF ZOÖLOGISTS. The female appears to be the heterogametic sex. Males may be heterozygous for any two of the four characters. Females carry the determiner for one only. The complete data upon which these statements are based are presented in the paper immediately following this one.

Secondary sex characters

In *X. helleri* the more striking external characters indicative of sex are these: the so-called sword tail which is an elongation of the ventral border of the caudal fin and appears only in males; the pelvic fins—in which the first ray is about one-third the length of the second in males and about two-thirds the length of the second in females. This fin is also long and pointed in males, rounded in females. The form index is larger for males than for females. The anal fin is strikingly different in the two sexes. In immature fish the anal fin has the same appearance in both sexes, but in the mature stages the anal fin of the male has assumed a more or less

[3] I have had a few specimens of two other varieties: one with a black spot just back of and above the distal end of the pectoral fin (plate 1, figure 6). This character is highly variable. The other variety is a "crescent" similar to that described for *X. helleri* (figure 7).

pencil-like structure and is carried parallel to the horizontal axis of the fish. One of the first indications of approaching maturity in males is a slight thickening of the third anal ray. Further, the cross-barring which gives each fin ray a somewhat segmented appearance and which is characteristic to some extent of the rays in all fins, extends into the proximal third of the third ray in the immature males. In females of all ages the cross-barring is confined to the distal half of the ray. Very often this extension of the pseudo-segmentation of the third ray to the proximal third is the first indication that the fish is a male. The third ray rapidly

TABLE 5

Age (in weeks) at which males of P. maculatus and X. helleri begin "transformation."

AGE	*P. maculatus*	*X. helleri*	AGE	*P. maculatus*	*X. helleri*
5	2	0	26	11	30
6	23	0	27	10	36
7	50	0	28	11	23
8	56	2	29	7	28
9	73	5	30	7	21
10	99	23	31	1	9
11	115	13	32	4	17
12	94	33	33	3	28
13	108	37	34	0	12
14	83	59	35	7	11
15	111	41	36	3	9
16	70	44	37	0	20
17	37	59	38	0	7
18	41	50	39	4	2
19	27	53	40	0	5
20	60	42	41	1	4
21	19	61	42	2	4
22	31	37	43	3	3
23	11	54	44	1	2
24	14	28	45	0	1
25	16	26	Means	14.7	21.8

increases in thickness and length and comes into intimate relation with the fourth ray. The entire fin appears to fold up fan-wise until it extends parallel to the caudal peduncle. The elongated portion of it consists of the modified third, fourth and fifth anal rays. The entire process from the beginning of the thickening of the third ray to the completion of differentiation requires about ten days as a rule, although the time may be extended indefinitely in the case of specimens we are calling intersexes. The modified fin functions as an intromittent or contact organ in the transference of sperm.

There is no such striking difference between males and females in *P. maculatus*. The sword is absent and the differences in the pelvic fins, while they are of the same character as in *X. helleri*, are somewhat less conspicuous. The transformation of the anal fin proceeds in males in much the same way as in *X. helleri*.

Concerning the finer structure of the anal fin of mature males there is considerable difference between *X. helleri* and *P. maculatus*, a fact noted for these and other members of the group by REGAN (1913) and used by him in diagnosing various species of these fishes. Further, there appears to be considerable variation in the finer details of structure within the species. Further description is reserved for the account of hereditary behavior of the generic hybrids.

Behavioristic differences, aside from mating reactions, also distinguish the sexes to some extent, but the differences are so elusive as to be difficult of exact description. In general, the males of *X. helleri*, especially, are more active, more 'nervous' and somewhat more easily disturbed or frightened than females.

The age at which the anal fin of males begins to undergo the transformation indicated on page 524 is subject to wide variation. In *X. helleri* the variation is from 8 to 45 weeks, the average being 21.8; in *P. maculatus* the range is from 5 to 44 weeks with a mean of 14.7. While we have adopted a standard routine for isolating males there is no doubt considerable error in estimating the exact day when a male is said to reach maturity. When young are born their record number is entered on a special calendar in such a way that every fish in every tank where there are immature animals, is examined once each week and the young males, if any are present are isolated. This weekly observation is continued until it is certain that only females remain in the original tanks. However, on several occasions we have been mistaken in diagnosing all of the fish remaining in a tank as females. In at least four instances after the final record of the sex ratio had been entered we later (4 to 28 weeks) found a male, in one case three, among the females. Probably these represent simply delayed transformations from the indifferent juvenile condition to the mature, although at such times one is reminded of the possibility of an inversion of sex.[4]

[4] Material for the study of the histological changes accompanying the approach of sexual maturity, and the possibility of an inversion of sex, was given to Doctor J. M. ESSENBERG in 1920. His findings ". . . decidedly favors sex-inversion in *Xiphophorus helleri* . . ." which ". . . takes place most commonly in immature fishes, but may occur in adult animals." This statement means, I take it, that inversion in adult animals is a possibility, even probability; not that inversion of females known to have produced young has been observed in this laboratory.

There is, then, an error of at least a week in the age recorded as that of attaining maturity. This, however, presupposes one's ability to detect males at comparable ages. The criterion we use is the appearance of the third ray of the anal fin. When this ray is perceptably thicker than the other anal rays (and if the cross-barring or pseudo-segmentation extends into the proximal third of the ray) the fish is isolated as a male. Both these changes appear gradually, and the latter can be detected sometimes several weeks before the ray shows any increase in diameter. Once the third ray begins to grow thicker, the changes usually proceed rapidly to completion. However, with very few exceptions, every brood of young reveals a few, varying from 0.1 to 50.0 percent, individuals isolated as males that remain for months in various stages of partial transformation, and some never become perfect males. Provisionally, we are calling these incompletely transformed individuals intersexes. Although a considerable amount of information has been obtained concerning the numerical proportions and ancestry of these individuals, nothing is known of the factors involved in their appearance or of their cytology and it is felt that further discussion would better await opportunity for their more complete study.

It is seen from table 5 that in the mean ages at which males of *X. helleri* and *P. maculatus* mature there is a difference of about 7 weeks. Although this is a large difference, in view of the large, and at present unavoidable, errors of determination or observation, we are not much tempted to make any further statistical analysis or even to attach too much significance to the numerical expression of the difference. Refinement of the routine methods of detecting and isolating will no doubt give data that can be treated statistically with some satisfaction. It should be said in this connection that the data on this point were obtained incidentally from

Briefly his conclusions are based on these observations: (1) The juvenile sex-ratio approximates 1 male to 2 females; (2) in about 50 percent of the young females studied by ESSENBERG regressive changes are seen in the ovaries. The follicle degenerates, the ovarian epithelium is resorbed, and the originally paired gonads—which remain paired in adult males and form a single structure in mature females,—give rise to a testis or testis-like structure bifurcated at the posterior end. (3) The occurrence in some individuals of the bifurcated testis just mentioned, which is regarded as an intermediate stage in the process of transformation of an ovary to a testis. (4) The anal fin of retrogressive females is much like that of the young male.

As regards the sex ratio of adult *X. helleri*, it should be said that the average excess of males (54.3 percent) for the entire population raised between 1918 and January 1924, is not as great as is indicated by the data available when ESSENBERG's observations were made. However, since the data used by him are from cultures that parallel those that furnished his material the sex-ratio given in his table III (73.3 percent males) should be used for comparison with the juvenile sex-ratio rather than the average for the entire population.

the records. We were primarily interested in securing virgin females and since we adopted the habit of recording the date when each male was isolated it was a simple matter to calculate his age at that time. Further, it is apparent on looking over the records that as our experience with the material increased our ability to detect males earlier, improved.

In *P. maculatus*, where certain sex-linked characters are introduced in the proper manner, the sex ratio can be determined a few weeks after birth. For example: White male × Rubra (or Nigra or Pulchra) female gives in F_1 Rubra (or Nigra or Pulchra) males and White females.

SEX RATIOS

While the two sexes in both genera do not depart widely from numerical equality, it is clear that in *X. helleri* an excess of males is characteristic; in *P. maculatus*, a slight excess of females. Since the complete pedigree records are to appear in the report immediately following this one and from which the variations in the sex ratios in the several progenies can be obtained, it will be sufficient here merely to state the total number of males and females for the two genera. For *X. helleri*, out of 2835 young born, 1126 males and 947 females were recorded, or 100 males to 84.1 females. The mortality here is 26.9 percent. From 6088 *P. maculatus* young born, there were recorded 1875 males and 2156 females, or 100 males to 109.4 females. Here the mortality is 33.79 percent. "Mortality" does not necessarily mean that all of the unrecorded fish died before reaching sexual maturity. Probably 5 percent are killed accidently with dip nets or in other ways in washing tanks. In a few instances among the 300-odd tanks in the laboratory, a progeny from which the males had been removed and which was of no particular interest save for the sex ratio, has been neglected until many of the fish remaining (supposedly females) had died. In these few cases we have of course recorded as female only those alive and definitely accounted for at the time of making the record. Again, probably one out of each 500 fish isolated as males, is a female, although this introduces no error except when such fish die in the male tanks before their presence there is detected. In this case we are erring in the direction of safety,—since we have to be sure of keeping all males out of the female tanks.

It will be seen that the fish that die or whose sex is not recorded are more than enough to bring the sex ratio to equality in both cases. But also it is clear that the averaging of all the figures has simply obscured the variations in individual progenies. For example in one brood of *X. helleri*

where 81 young were born there were recorded 60 males and no females. Another brood of 40 young gave 35 males and 1 female. In another case, from a total of 116 young born there were recorded 19 males and 71 females. For *P. maculatus* the variations are not so large and it would not be unreasonable to suppose that the mortality is sufficient to account for the slight preponderance of females.

The relative susceptibilities of males and females to conditions that kill very slowly may throw some light on the sex ratios. Both sexes in both genera have been tested in dilute solutions of KCN, ethyl alcohol, HCl, NH_4OH, high temperature, and lack of oxygen. In all cases, with some individual variation, the males die sooner than females. These facts, while they may furnish a basis for explaining the slight excess of females in *P. maculatus*, also tend to indicate a real preponderance of males in *X. helleri*. It must be said, however, that, as far as one can judge, the tanks rarely get in a condition toxic enough to kill the fish.

Further than mentioning possibilities it does not seem worth while just now to speculate on the explanation of the variable sex ratios. I have no doubt that lethal factors of some sort will be called upon and very likely they can be made to account for the results. If one such factor will not suffice several more may be assumed to operate. Unfortunately there do not appear to be, or perhaps better, I have not discovered, enough linked or other characters to make an experimental test of a hypothesis of this sort,—even if it meant anything after making the test. Fish fanciers have told me repeatedly of seeing females turn to males (during the course of several weeks) in *X. helleri* and there are references in the literature to such reversals of sex in allied genera of fishes. ESSENBERG (1923) has offered some histological evidence that complete reversal of sex in at least a part of the young of *X. helleri*, is normal to the species. And it may well be that some unstable condition with reference to sex is responsible for the irregular sex ratios. Although we have been continually on the watch for such a sex reversal, we have never seen such a transformation in a fish that had produced young as a mother. However, we have in formaldehyde a fish that, according to its owner, like CREW'S (1923) hen, functioned at one time as a mother and later as a father of young. We had the fish alive in our tanks for some time but never obtained young from it. It had the secondary sex characters of a male and the general body shape of a female.

Considering the fitness of the material for studies in experimental evolution it may be said that the animals are readily, though somewhat laboriously, handled in the laboratory. Platypoecilus, especially, is

variable enough and exhibits sufficient differentiating characters to make possible some factorial analysis. One rather infers from the taxonomic literature that the whole group in nature is rather variable, perhaps unstable, or in process of "species making." The fact that they are aquatic makes possible certain types of environmental control impossible for air-breathing types. Their viviparity tends to insure a fairly stable and uniform environment for the early developmental stages, but makes impossible the environmental control of these stages except within narrow limits. The life cycle is such that one can easily obtain two generations in a year and can hope to raise a reasonably large number of young from each female. There is very little sterility or failure to breed within the species. It is not possible yet to say just how difficult cytological study of these forms is going to be, but the indications are that it will be difficult enough. Certainly the chromosomes are small and numerous, and further we have had great trouble thus far in finding in the germ cells any mitotic figures to study. The fact that the two genera give fertile hybrids makes it seem just now, in addition to studies of environmental effects, that work on the rôle of hybridization in species making in this group, will prove the most fruitful line of attack. Several new types have appeared among the hybrids, that differ conspicuously from either of the parent forms, and the indications are they will remain constant. As regards sterility in the hybrids it appears, curiously enough, that the majority of F_1 hybrids are fertile, while the majority in F_2 and F_3 are sterile. Also many of the offspring resulting from a back-cross of the F_1 hybrids to either of the parent forms do not breed. The data thus far obtained on the hybrids are now being prepared for publication and it is expected that they will appear soon after the report on the sex-linked multiple allelomorphic series in Platypoecilus.

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GAMETOPHYTIC INHERITANCE IN SPHAEROCARPOS
I. INTRACLONAL VARIATION, AND THE INHERITANCE OF THE TUFTED CHARACTER

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INTRODUCTION

Various writers have discussed the advantages to be hoped for from the genetic study of haploid, as distinguished from diploid, organisms, with the latter of which such investigation has thus far been mainly concerned. Chief of these advantages is the opportunity of observing in a haplont virtually the immediate genetic effects of meiosis. It is to be expected, therefore, that the ratios obtained in the study of haplonts will be simpler than those dealt with in the study of diplonts, the difference in favor of the former being more marked the greater the number of pairs of contrasting characters under investigation; if current genetic conceptions

represent an approximation to fact, the complications of heterozygosis, dominance and recessiveness would disappear; and such interrelations between characters as are expressed in linkage and crossing over would be more readily recognizable. Sphaerocarpos, like a few other bryophytes, offers the possibility of a special advantage resulting from the remaining together of the four spores formed by the division of a single spore mother cell, an advantage which has been made use of in the study of sex inheritance. However, as will appear, practical difficulties have interfered with the full realization of this particular advantage in the studies here reported, although cultures now in progress promise results more satisfactory in this respect.

Studies of inheritance by haplonts, or supposed haplonts, among the algae, include those by PASCHER (1916, 1918) of the results of crossing species of Chlamydomonas; by TRANSEAU (1919) of what appeared to be hybrids between distinct species of Spirogyra; and by ERNST (1917, 1921) of the relations between parthenogenetic and sexual strains of *Chara crinita* and the inheritance of sexual characters by the offspring of sexual plants. It is true that the listing of PASCHER's results in this category involves the assumption that chromosome reduction occurs in the germination of the zygote of Chlamydomonas, an assumption not yet proved, although it is rend ·ed highly probable by DANGEARD's (1898) statement that no reduction occurs in the gametangium of Chlorogonium, and by ZIMMERMANN's (1921) discovery of meiosis in the germinating zygote of Volvox.

It has been shown that the sexual characters (or better, perhaps, the characters inducing or preventing gametic union) of certain heterothallic fungi are inherited by haplonts in a manner that suggests a segregation, during meiosis, of the physical bases of these characters. Evidences of such segregation are supplied by BLAKESLEE's extensive work (résumé in his paper of 1920) on various Mucorineae, and by the studies of EDGERTON (1914) on strains of Glomerella and of KNIEP (summarized in 1919 and 1922) on species of Ustilago and of Hymenomycetes. BURGEFF (1912, 1915) has also found that distinctive vegetative characters of certain races of *Phycomyces nitens* are transmitted in a manner analogous to that marking the inheritance of sexual characters. It must be remembered, however, that, as for Chlamydomonas, so for the Mucorineae and the Ustilaginales, the time of chromosome reduction is not yet conclusively demonstrated; although in the latter group there is little doubt that meiosis typically occurs in the germinating brand-spores.

Somewhat more extensive is the knowledge gained of the inheritance of sexual characters in bryophytes, particularly in heterothallic (dioicous) species. A considerable number of bryophytes are found to be strictly heterothallic, in the sense that a haploid plant or clone arising from a single spore is invariably, so far as observation has shown, of a single sex; by no methods yet attempted has such a plant been induced to change its sex or to give rise by vegetative means to a plant of opposite sex. The list of heterothallic species includes, among the Hepaticae, *Marchantia polymorpha* (NOLL, reported by SCHULTZE 1903; BLAKESLEE 1906), *Conocephalum conicum* (BLAKESLEE 1908), two (or three) species of Sphaerocarpos (DOUIN 1909, ALLEN 1919), and *Riccia Curtisii* (MCALLISTER 1916); and, among the Musci, one or more species each of Barbula, Bryum, Ceratodon, Mnium (MARCHAL and MARCHAL 1906, 1909), Macromitrium, Schlotheimia, Trismegistia (FLEISCHER 1902-1904, 1920), and Splachnum (SCHWEIZER 1923). WILSON (1915), however, has observed archegonium-like structures borne on an otherwise male haploid plant of *Mnium hornum*, a species found by the MARCHALS to be heterothallic. This isolated observation may suggest the possibility of the existence of distinct heterothallic and homothallic races within the same species. For most of the species above mentioned, it has been shown that a single sporophyte produces spores bearing respectively male and female potentialities. The statement as to the strict heterothallism of Sphaerocarpos is confirmed by the observation of some thousands of cultures of *S. Donnellii* and of a smaller but considerable number of cultures of *S. texanus* that have been grown at the UNIVERSITY OF WISCONSIN during the past eight years.

In *Sphaerocarpos Michelii* (or possibly *S. texanus*, or both) (DOUIN 1909) and in *S. Donnellii* (ALLEN 1919), the four spores resulting from the division of a single spore mother cell give rise regularly to two female and two male gametophytes; and in *S. Donnellii* (ALLEN 1917, 1919) and *S. texanus* (SCHACKE 1919), the distribution of sexual potentialities is correlated with that of two visibly different homologous chromosomes.

In the homothallic *Funaria hygrometrica*, on the other hand, CORRENS's (1920) results, confirmed and extended by unpublished work of Miss MABEL BROWN, show that, contrary to the evidently erroneous conclusions of COLLINS (1919, 1920), there is no segregation of sexual potentialities as between "female" and "male" branches, or even between the cells of the respective sex organs. Agreeing with this conclusion are the results of regenerations from stems and leaves of *Amblystegium serpens*, also a homothallic species, briefly reported by the MARCHALS (1909).

The only study thus far reported of the inheritance of other than sexual characters by the haploid generation of bryophytes, apart from my own work on Sphaerocarpos, is that of WETTSTEIN (1923). He describes the outcome of crosses between haploid, as well as between diploid, and between haploid and diploid, gametophytes of *Funaria hygometrica* differing in respect to several characters, both gametophytic and sporophytic. WETTSTEIN's work also included crosses involving Funaria and species of Physcomitrium and Physcomitrella.[1]

The one published report of work in this category dealing with a pteridophyte is by Miss ANDERSSON (1923). She finds that the spores from a single sporange of a variegated race of *Adiantum cuneatum* give rise to prothallia of two types, respectively green and pale green.

Some observations have been made of gametophytic characters in angiosperms whose distribution seems to result from a meiotic segregation. The production within the same anther of pollen grains of diverse types, differing in size or structure, in the presence and absence or form of starch granules, or in the chemical nature of other reserve foods, is recorded in cotton (WATT 1907), in various forms and crosses of Oenothera (RENNER 1919 a, b), in rice (PARNELL 1921), in maize (DEMEREC, reported by JONES 1923),[2] and in one group of Datura mutants (BLAKESLEE 1924). GEERTS (1909) found that often, in *Oenothera Lamarckiana*, only two pollen grains of a tetrad develop normally, the others degenerating. A similar observation in one clone of Rhododendron is reported by BELLING (1921). Evidence of a differential rate of growth of tubes from genotypically different pollen grains produced in the same anther is reported for Oenothera (RENNER 1919 a, b; HERIBERT-NILSSON 1920), Melandrium (CORRENS 1917, 1918, 1921; UBISCH 1922), Rumex (CORRENS 1922), and Datura (BUCHHOLZ and BLAKESLEE 1922, 1923). BELLING (1914) reports that, in certain Stizolobium crosses, about half the microgametophytes and about half the macrogametophytes abort, the remaining gametophytes of either sex developing normally.

With these studies of inheritance in haploid plants should be mentioned those dealing with the genetics of haploid males among the Hymenoptera, developed from parthenogenetic eggs. In this class belong the work of CUÉNOT (1909) and NEWELL (1915), as well as the less well controlled

[1] A more extensive account of WETTSTEIN's studies (Zeitschr. indukt. Abstamm. u. Vererb. 33: 1-236, 253-257, 1924) has appeared since the writing of the present paper.

[2] DEMEREC has since reported his own results (Amer. Jour. Bot. 11: 461-464, 1924). Simultaneously with his paper appeared one by R. A. BRINK and J. H. MACGILLIVRAY (Amer. Jour. Bot. 11: 465-469, 1924) announcing independent, but essentially similar observations.

observations of older authors, on bees, and the more extensive studies of WHITING (1921 a, b, c, 1923) on the wasp Hadrobracon.

From the beginning of my work with Sphaerocarpos, the possibility has been in mind of the appearance in the gametophytes of genotypic differences other than those which distinguish the sexes. Of numerous clones isolated and studied from this point of view, several have been found to differ so markedly from what may be considered the typical condition as to afford promising material for genetic study. It was in 1918 that the aberrant form whose behavior is described in the present paper was first observed and isolated. Some of the results of its study and of that of other forms discovered later have been reported at various society meetings from 1919 to the present year, and brief notes of some of this work have been published (ALLEN 1923 a, b).

The general methods used have been described (ALLEN 1919). The work has been carried on with the assistance of Dr. H. W. RICKETT during 1920-1921 and 1921-1922; of Dr. A. M. SHOWALTER in the summer of 1921; and of Mr. J. A. LOUNSBURY during 1922-1923 and 1923-1924. The drawings accompanying the paper were made by Miss IRMA HAAK and Miss BETTY THORKELSON. This assistance has been made possible by grants from the research fund of the UNIVERSITY OF WISCONSIN.

The drawings were made with the aid of a binocular microscope and an ABBÉ camera lucida. The drawings of female plants, allowing for reduction in engraving, are magnified about six and one-half times; those of male plants, about eight and one-half times.

THE CHARACTER OF A TYPICAL FEMALE CLONE

It was noted in a previous paper (ALLEN 1919) that the gametophytes of *Sphaerocarpos Donnellii* Aust. display a considerable variety of structure when subjected to varying environmental conditions. This is shown in the characteristically different forms of plants of either sex when found in their native habitat and when grown in the greenhouse (ALLEN 1919, figures 1 to 4). Noticeable differences often appear between plants grown under as nearly as possible uniform conditions,—as, for example, between those in the same pot; and much more conspicuous variation occurs in response to changes of some magnitude in cultural conditions.

Preliminary to any genetic study of structural differences, it is evidently important first to determine approximately the range of possible variation among plants of the same genetic constitution. Sphaerocarpos lends itself

readily to a study of this nature, since from a single plant, or even from a small part of a plant, a clone of any desired size may be obtained by isolating the plant or part under conditions favorable to growth. By means of the growth and branching of the plant, and of the separation of the branches as independent plants[3] by the death of the older parts, as well as by means of the production of regenerated shoots, which, as RICKETT (1920 b) has reported, occur frequently in cultures, a clone covering the surface of the soil in a three-inch pot may develop in a few months. Such a clone may be divided, giving as many separate cultures—or, as they will be referred to hereafter, sub-clones—as may be desired.

For the purpose of such a study, a female clone (19.24) was selected which for present purposes will be referred to as "typical." Obviously such a selection is an arbitrary one, and further study may show that a clone differing from this one (showing, perhaps, for example, a smaller proportion of irregularities) would serve better as a basis of comparison. However, the clone here considered well represents the common form of the species, so far as can be determined from the examination of greenhouse material. Its history is as follows:

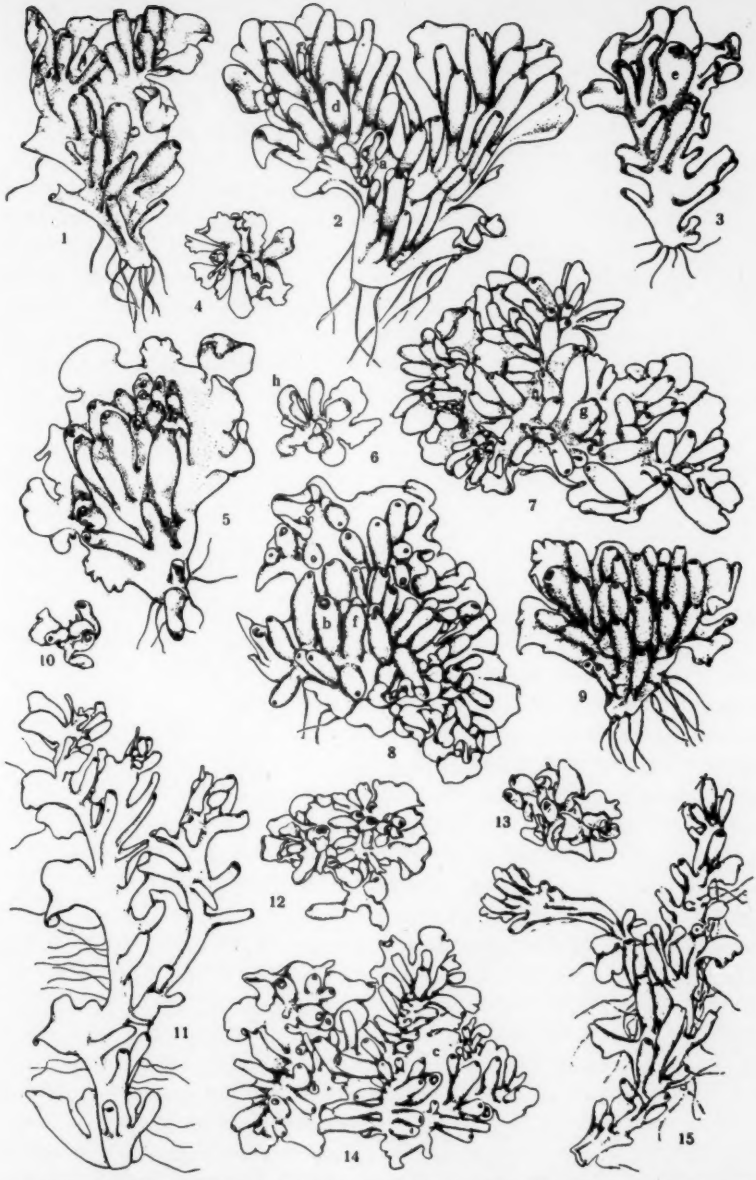
Some of the spore tetrads from a sporophyte that appeared in a culture (containing plants of both sexes) of *S. Donnellii* received February 16, 1916, through the PLANT STUDY COMPANY from Miami, Florida, were sown on soil June 28, 1916. In the culture that resulted from the germination of these spores the male plants gradually disappeared, as is usual in such mixed cultures. On May 24, 1918, this culture, now apparently purely female, was transplanted to a pot with a male culture derived from the germination of two spores of (probably) a single tetrad taken from a sporophyte that appeared also in a culture received February 16, 1916, from Miami. The results as to sex of the germination of the spores from this particular sporophyte are included in a previous paper (ALLEN 1919, table I). The pot containing the female and male plants thus derived was several times flooded with sterilized water, and the tetrads from one sporophyte resulting from the fertilization thus induced were sown, one tetrad to a pot, December 27, 1918. The results of this sowing are included in those reported on pages 299 and 300 of the previously cited paper. The germination of one spore (the only one of its tetrad that germinated)

[3] It is not easy in practice to distinguish between a "branch" and a "plant." Any branch may, sooner or later (the exact time depending upon the rate of death and decay of the older tissues), become an independent plant.

resulted in the female plant that was the starting-point of clone 19.24, which clone, as it developed, has been at various times divided.

The plants and parts of plants shown in figures 1 to 15 (all drawn to the same scale) represent fairly well the range of variation that has been observed within this clone in plants bearing no sporophytes. A glance at the figures shows that there are marked differences in size of all conspicuous parts—including lobes, involucres and rhizoids. There are differences also in the degree of crowding or separation of the lobes, in the spacing of the involucres, and in the abundance of rhizoids. The latter point is not well shown in the figures, because these are drawn in dorsal view and because some of them (those showing no rhizoids) were drawn while still in place upon the soil. The general appearance of the plants growing vigorously under generally favorable conditions is well represented by figures 2 and 9. Plants growing isolated or at the margin of the culture are likely to produce larger lateral lobes. Figure 8 represents a plant from a culture that had been allowed to become comparatively dry. One effect was a dwarfing of the involucres, particularly in the younger part (the upper part in the figure), although the very smallest involucres here shown were young and had not yet attained their full growth. A similar condition, even more marked as to the dwarfing of involucres, characterizes the plant shown in figure 14. The latter plant grew under very crowded conditions, which resulted in its being forced upward and thus probably in its receiving a lessened supply of water and soil nutrients. Even more striking instances have been observed of a dwarfing of involucres, apparently as a result of crowding and of poor nutrition, in old cultures.

Figures 11 and 15 are of plants from a culture that was kept for some weeks in a thoroughly moisture-saturated atmosphere. In this condition the branches grew vertically, instead of approximately horizontally as is usually the case when the air is materially below the saturation point; abundant long rhizoids were produced; and the thallus grew disproportionately in length, this growth resulting in a marked increase in distance between the lateral lobes as well as between the involucres. Plants of this form are suggestive of those described and figured by CAMPBELL (1896) for a Californian species of Sphaerocarpos grown under glass in a humid atmosphere; he found, too, that plants growing spontaneously in unusually moist or shaded places showed a tendency toward a similar habit of growth. RICKETT (1920 a) has obtained like results in plants of *S. Donnellii* grown under conditions of excessive moisture, and his figure 70 is very like figure 11 of the present paper. Plants growing



FIGURES 1 to 15.—Plants and branches of a typical ♀ clone (19.24) of *Sphaerocarpos Donnellii*. ×6.5.

submerged in water (RICKETT 1920 b, figures 24, 25) show still greater elongation and very scattered involucres.

The lateral lobes vary from flat to much curved or crispate, and their outlines range from entire to undulate or one- or several-times incised. Considerable variation appears in the shapes of the involucres, and particular attention has been paid to these because it is the involucres that show the most marked irregularities in the atypical form to be described later. The vast majority of involucres on plants growing under ordinarily favorable greenhouse conditions have the general appearance of those shown in figures 2 and 9. The shape is that of a cylinder somewhat constricted at base and apex, with a rather small terminal mouth; minor differences in length, diameter, degree of basal and apical constriction, and size of mouth are manifest among the mature involucres borne on any plant. The hood-like structure at a, figure 2, is apparently a dorsal lobe (an infrequent but not extremely rare type of structure) which has already produced one (still small) involucre on its dorsal surface.

For the most part, marked divergences from the usual shape of involucre are obviously due to unfavorable conditions of various sorts. Mention has been made of the dwarfing effect upon the involucres of drying or of crowded growth. The involucres of plants exposed to a relatively dry atmosphere tend to approach the ovate-ellipsoid form, truncate at the apex, which characterizes those of plants in their native habitat (compare the involucres shown at the left in figures 8 and 14 with HAYNES 1910, plate 29, figure 1, and ALLEN 1919, figure 1). Poor nutrition and a lack of moisture sometimes check the development of involucres, which then remain comparatively short, with wide-open or even flaring mouths. Involucres of this nature are shown in the upper portion of figure 12, which, however, probably represents a branch resulting from regeneration (see below). On one occasion a young branch was seen in clone 19.24 that was light green in color and bore several compressed and more or less deformed involucres. Similar branches have been occasionally observed in other otherwise typical female clones; they represent the nearest approach seen in branches of such a clone to the "tufted" condition described on a later page. The irregular forms of the involucres on such branches seem to be due to crowding; the light color is probably also a result of crowding which has forced the branch to grow at some distance from the substrate, thus reducing its supply of water and mineral nutrients.

Other more or less frequent deviations from the usual condition are a curvature (see especially figures 7 and 14), due sometimes to crowding and

sometimes apparently to the effect of other environmental conditions upon the rate of growth on different sides; especially large mouths, sometimes irregular in shape (b, figure 8), and now and then so large that there is little or no apical constriction of the involucre (several involucres shown in the upper part of figure 1); a displacement of the mouth to a lateral instead of a terminal position (b, figure 8; c, figure 14); and an unequal growth in diameter at different levels of the involucre, resulting in a swollen appearance near the base (c, figure 14), in the middle portion (d, figure 2), or toward the apex (e, figure 3), or in an hour-glass shape (f, figure 8). Sometimes the most extensive growth is local, resulting in a pouched appearance (g, figure 7). Another occasional occurrence is that of an appendage or outgrowth from the outer surface of the involucre. These appendages, in the female, ordinarily take the form of vertical plates one cell in thickness; very rarely such an appendage extends nearly or quite the length of the involucre. In figure 10 is shown an involucre with two appendages; it is rare, however, in clone 19.24, to find an involucre bearing more than one appendage, and even single appendages are infrequent. Other clones have been found, a larger proportion of whose involucres bear such appendages, and others in which, so far as numerous observations have disclosed, involucral appendages are quite lacking. The oldest involucres of a branch are sometimes large and more or less aberrant in shape, being variously swollen, knobby or pouched, or having somewhat lateral and irregularly shaped mouths. Such appearances are most common in the older, slowly growing cultures, and less frequent in cultures that are growing vigorously. Apparently, under conditions that favor a slower and more long-continued development of individual involucres, these tend in their later history toward an irregular fashion of growth.

The plants represented in figures 1 to 15 were selected for the purpose of showing as many as possible of the variations observed within a single clone. The impression given by these figures is likely, therefore, to be that the proportion of variations is greater than is really the case. As a matter of fact, in a healthy culture practically all the plants are substantially similar in appearance, and careful searching is necessary to discover involucres or other parts that diverge markedly from the conditions shown in figures 2 and 9. Figure 36, a photograph of a portion of clone 19.24, gives a good notion of the generally uniform appearance of such a culture. The differences in size between the involucres shown in this photograph largely represent differences in age.

Figure 16 shows a plant of this clone with immature sporophytes, the presence of which is evidenced by the much-swollen bases of certain

involucres; the plant of the same clone shown in figure 17 bears a number of mature sporophytes. In the latter case, the black color of the spore tetrads shows through the capsule walls, the calyptras, and the bases of the involucres. The development of a sporophyte not only results in such basal enlargement of the involucre as is necessary to accommodate the spherical capsule with its calyptra; the development of all parts of the

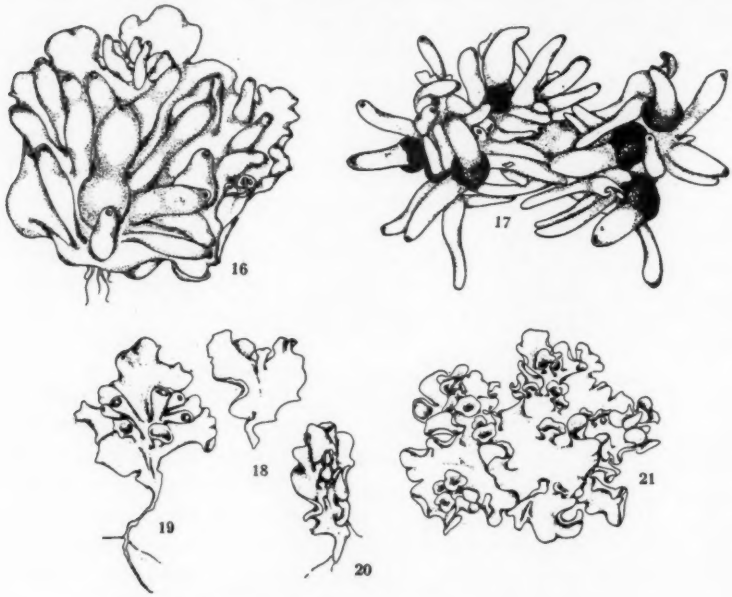


FIGURE 16.—Female plant bearing immature sporophytes.

FIGURE 17.—Female plant bearing mature sporophytes. Both from clone 19.24. ×6.5.

FIGURES 18, 19.—Female sporelings, resulting from the fertilization of eggs of typical ♀ plants (clone 19.24) by antherozoids of typical ♂ plants (clone 19.27). ×6.5.

FIGURE 20.—Male sporeling of the same ancestry. ×8.4.

FIGURE 21.—Female sporeling, from a cross between a tufted ♀ and a typical ♂ (mating 3). The clone (20.304) developed from this sporeling is classed as 22 percent tufted. ×6.5.

involucre seems to be stimulated, so that involucres enclosing capsules very commonly stand out as conspicuously larger than their empty neighbors.

It seems to be a regular rule in the development of a female gametophyte from a spore that the earliest involucres produced are not only smaller than those formed under ordinary conditions by a mature plant, but are

also of different shape. Figure 18 shows a sporeling with its first-formed involucre. The involucre (in the upper part of the figure) has its mouth turned away from the observer, but it is plainly vase-shaped, with a large and flaring margin. The sporeling shown in figure 19 has produced seven involucres; the oldest two have the same vase shape, and the mouth of one (probably the second oldest) is irregularly margined; the third, fourth, and fifth (in the middle part of the figure) are somewhat constricted apically but have large mouths; the sixth and seventh approach more nearly the characteristic form. Both these sporelings were the result of a mating of clone 19.24 and the typical male clone (19.27) described on a later page.

These juvenile characteristics (small involucres, with wide or flaring mouths and other deviations from the common form) occur also on regenerated shoots. The branches shown in figures 4 and 13 are certainly, and those represented by figures 6 and 12 are probably, the results of regenerative growth. (Obviously it is impossible to be certain of the source of a regenerated branch after its separation from the parent plant, which may occur very early.) All these branches bear small involucres of variously irregular shapes. The fully formed involucres of figure 4 are all of the flaring type. At h in figure 6 is shown perhaps the most marked deviation of its sort that has been observed in this clone; the involucre is hood-like, the mouth being laterally placed and so elongated vertically as to extend nearly to the base of the involucre.

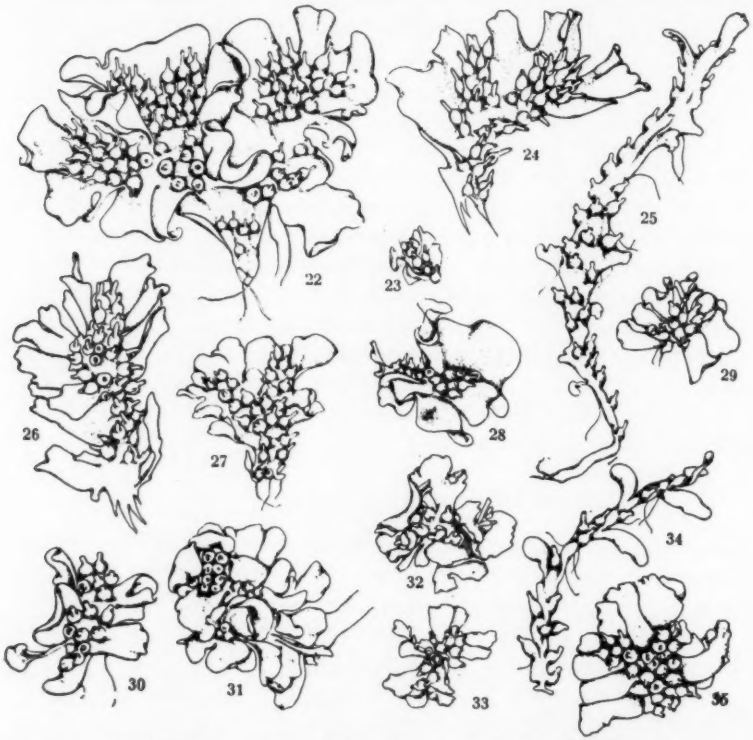
THE CHARACTER OF A TYPICAL MALE CLONE

The selection of a male clone to be considered as "typical" is subject to the same reservations noted in the discussion of a female clone. The male clone (19.27), plants of which are shown in figures 22 to 35, resulted from the germination of a spore of a tetrad borne in the same capsule as was that which gave rise to the female clone 19.24. Figures 22 to 35 are all drawn to the same scale, which, however, is somewhat larger than that used for the figures of female plants.

In their range and general nature, the variations within this clone are comparable with those observed in clone 19.24. Naturally, since the parts of the male plant are correspondingly smaller than those of the female, differences of the same proportional magnitude are less conspicuous in the male and are more easily overlooked. Apart from the involucres, what has been said of the nature of variation in the female applies equally to the male. As in the female, the spacing of the involucres and, in con-

sequence, their number on a given length of the thallus, are variable features. Growth in a saturated atmosphere affects male plants, as to habit and appearance (figures 25, 34), essentially as it does female plants.

Variation in the structure of antheridial involucres is evidently, as in the case of the archegonial involucres, due mainly to environmental differences. Variation appears in the size of the involucre as a whole; in the proportional diameters of base and neck; in the form of the neck, whether



FIGURES 22 to 35.—Plants and parts of plants from a typical ♂ clone (19.27). ×8.4.

straight or curved, and especially in its length. Differences in these respects will be readily noted in figures 22 to 35. Occasionally the neck is much reduced or even quite lacking; but such extreme conditions are rarely seen except on regenerated shoots, or in unhealthy cultures the development of whose involucres has evidently been retarded. Rarely an exposed antheridium is seen, not only the neck but part of the base

of the involucre being absent; in such a case the involucre may be saucer-like or may resemble a dorsal lobe of the thallus subtending the otherwise naked antheridium. Instances of such involucres appear in figures 23 and 33, which, however, represent what seem to be regenerated shoots. In the rare instances in which exposed antheridia have been seen on other than regenerated shoots, the possibility exists that the missing parts of the involucre had been destroyed, perhaps by insects. Some differences have been observed in the sizes of the mouths of involucres, but in this respect also marked deviations from the ordinary condition have been found only on regenerated branches.

Another occasional divergence from the usual form of involucre consists in an external appendage, growing usually from the basal part of the involucre, but sometimes from the neck. Several such appendages are shown in figures 26 and 35. The plants represented in these two figures were selected because their involucres showed an unusual number of appendages. Really, in this clone, as in the female clone described, the occurrence of such appendages is very infrequent. Sometimes the appendages, in the male, as in the female, are plate- or scale-like and one cell in thickness. Most commonly, however, their form on male plants is that of a slender, one- or few-celled hair-like protuberance.

Slow and long-continued growth of male thalli does not result in irregularities in involucral form comparable with those observed under similar conditions in female plants; it produces at most an extreme lengthening of the necks of antheridial involucres. This difference between male and female, like that noted below in the development of the earliest-formed involucres of sporelings, seems to be an expression of a general tendency, in the clones here under discussion and in their descendants, toward a greater range of variation in females than in males.

The early involucres of young typical male sporelings do not show unusual forms comparable with those characterizing the first few involucres borne on female sporelings. Occasionally, some of the earliest antheridial involucres are slender throughout their length,—lacking the enlarged base and apparently also the enclosed antheridium. A typical male sporeling (from a spore of a sporophyte resulting from the mating of clone 19.27 and clone 19.24) is shown in figure 20. The first involucre (nearest the lower end of the figure) has the slender shape referred to; the slender involucres seen on the younger parts of the thallus are probably not fully grown.

Regenerated male shoots, however, show about as large a proportion of atypical involucres as do regenerated female shoots. Consequently,

cultures in an unhealthy condition, in which regeneration is going on abundantly from various parts of the thallus, present a relatively large proportion of involucres of unusual forms. Figures 23, 29, 32 and 33 represent plants (or branches) from such cultures. As already noted, one can not always be certain that a particular shoot, unless it is very small, is the result of regeneration; but there is little doubt that such was the origin of the plants shown in these figures. Various involucres of these plants show the characteristic irregularities. Most of the involucres are small and short-necked; involucral appendages are numerous; some involucres (especially the two near the lower edge of figure 29) have wide or flaring mouths; and in figure 23 (near the lower edge) and in figure 33 (about the middle) are shown two exposed antheridia, whose involucres are reduced to saucer-like structures that apparently do not even quite enclose the bases of the antheridia.

THE CHARACTER OF A TUFTED FEMALE CLONE

As the previous descriptions indicate, a considerable range of variation is possible within a single clone. Among the forms that have appeared in cultures of *S. Donnellii*, however, are some whose marked divergence from ordinary conditions suggested a genotypic basis. In so far as this suggestion has been supported by later study, it indicates that, as might be expected, the species in question is in some measure a complex of hereditarily different strains or races. It is evident that a suspicion of genotypic difference can be nothing more until extensive study has been made of the results both of vegetative multiplication and of sexual reproduction.

The racial character particularly considered in the present paper is referred to as "tufted." It was first observed in a culture (R35) of plants sent from Sanford, Florida, by Mr. SEVERIN RAPP and received on February 23, 1918. In June of the same year, several female plants in a group near one edge of the culture were observed to be of noticeably aberrant form. On June 29, a number of the atypical plants were transplanted, each to a separate pot, these plants becoming thus the starting-points of new clones.

The characteristic that first attracted attention to these particular plants was the presence of upright lobe-like structures growing from their dorsal surfaces and apparently replacing the involucres borne by typical plants. The dorsal lobes much resembled the lateral lobes regularly borne by the thallus, which are especially well developed under greenhouse conditions. The presence of the crowded dorsal lobes gave the

plants a tufted appearance, suggesting the name applied to this form.

Further study of the original tufted plants and of the clones derived from them has shown that as a rule a branch of such a clone bears, in addition to the lobes referred to, involucres approaching in form more or less closely those of typical clones. Sometimes a dorsal lobe is curved so as to resemble an involucre split on one side to its base. The edges of other curved lobes are united at their bases, and those of others for varying distances from the bases upward. There are thus found all possible gradations from flat or slightly curved lobes to structures which are plainly involucres, although many of the latter vary, in ways to be described more fully below, from involucres of typical form. Not infrequently, however, strictly typical involucres appear on a plant or branch bearing chiefly atypical structures. Dissection shows that regularly at the base of each dorsal lobe, transitional structure, or involucre, is an archegone; and the archegones have been shown by fertilization experiments to be functional about as regularly as are the archegones of typical plants. It is evident, then, that all the dorsal structures described are to be considered as involucres the structure of most of which deviates in various degrees from that of typical involucres.

One of the clones whose origin has been described (R35G),—including a considerable number of sub-clones derived from some of its constituent plants,—furnishes the material for the following description. What will be said applies, however, except for differences in the proportion of atypical structures appearing, to other tufted female clones derived from the same and from other original cultures.

It was soon observed that, among the branches produced in this clone, some are not distinguishable from those of a typical clone—that is, they bear involucres of the ordinary type. Other branches bear varying proportions of typical and atypical involucres (including among the latter the dorsal lobes); and at all times there have been present branches bearing predominantly atypical involucres, which branches were thus about as aberrant as the plant with which the clone began. Not infrequently a considerable patch within a culture of the clone consists of strictly or predominantly typical branches—a "typical" branch being one whose involucres vary only within the range characteristic of a typical clone; other patches of the same culture consist of tufted branches. But an intermingling of tufted and typical branches is also common; and at any time a tufted branch may arise in a typical part of the culture, or *vice versa*. Since, as appeared more clearly from experiments to be de-

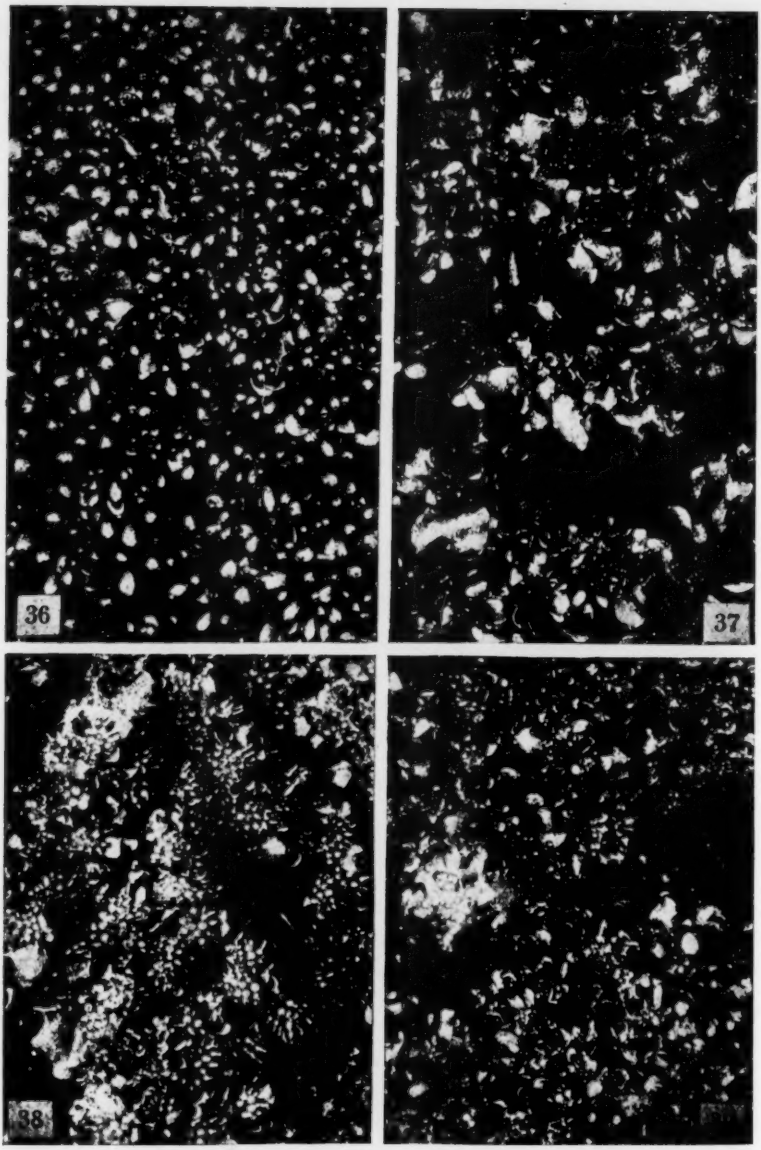


FIGURE 36.—Portion of a typical ♀ clone (19.24).
FIGURE 37.—Portion of a tufted ♀ clone (20.77).
FIGURE 38.—Portion of a typical ♂ clone (19.21).
FIGURE 39.—Portion of a tufted ♂ clone (20.107). ×5. Photographs by Mr. M. E. DIEMER.

scribed below, the tufted and the typical branches of a given clone are genotypically alike, it would seem that the marked phenotypic differences must be due to environmental influences. No light has yet been thrown on the nature of these influences by the intentional and unintentional subjection of this and similar clones to varied conditions. At all times, any sub-clone consisting of more than a few branches has included branches of tufted, typical, and intermediate character. While the proportion of tufted and typical branches within a sub-clone has varied from time to time, no relation has been found between such variation and the conditions of moisture, temperature, illumination, or constitution of the soil, or the presence of contaminating organisms (such as fungi or blue-green algae). Nor has any relation been observed between the proportion of tuftedness and the age or vigor of growth of a particular culture.

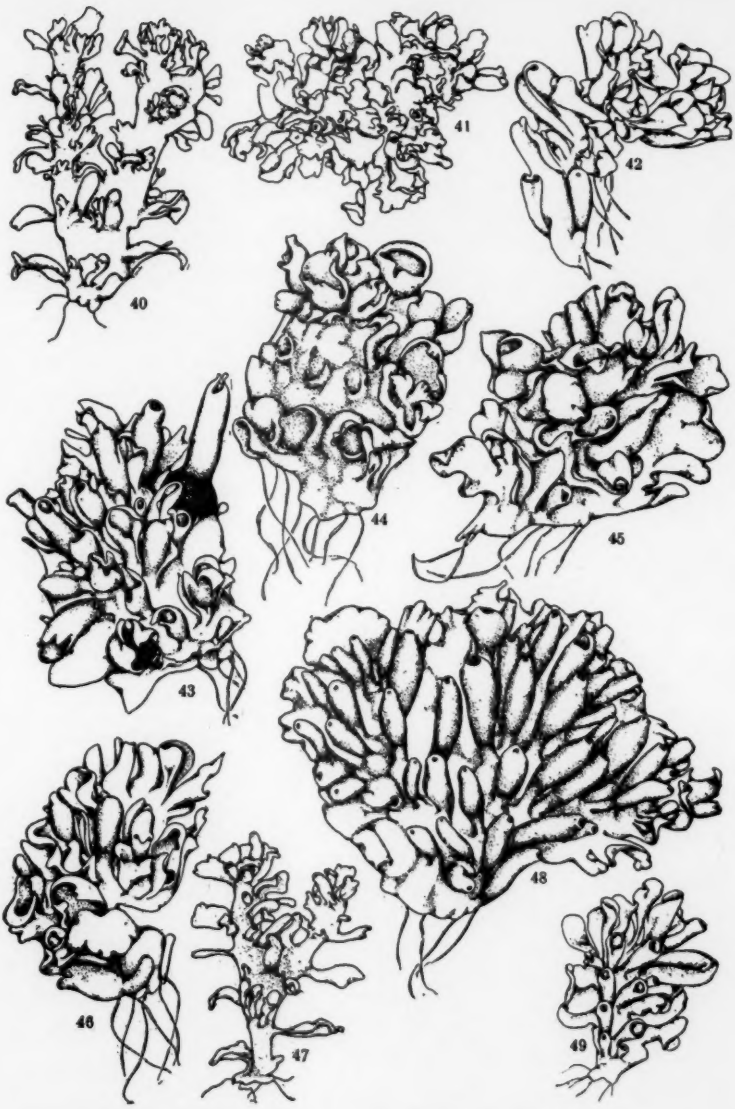
A characteristic that further justifies the term "tufted" consists in the very frequent tendency of the aberrant branches to grow upright, whereas the typical branches, except in a very moist atmosphere, ordinarily grow about as nearly horizontally as the available space allows. The upright habit of growth results in a greater crowding of the involucres (of whatever form) on a tufted branch, since they also tend to grow vertically. It results also, in a culture composed largely of tufted branches, in the general appearance of being made up of separated plants or tufts of plants, in contrast to the matted appearance characteristic of a typical female culture. These upright branches are frequently lighter in color than the typical ones, a difference which is probably connected with the lessened supply of water or nutrients resulting from the increased distance of their growing parts from the soil. In a culture with intermingled typical and tufted branches, the latter are often recognizable by the contrast of their light-green or yellowish color with the dark green of the typical branches. A similar lighter color of individual branches or of their involucres is, however, as already noted, occasionally seen in a crowded culture of typical plants.

The attempt has been made from time to time to measure the degree of tuftedness of this clone by estimating the proportion of the branches present which could be considered strictly tufted, as distinguished from intermediate and typical branches. For this purpose, a branch is considered as tufted if a decided majority of its involucres show markedly aberrant forms. Evidently such an estimate can be only approximate, and estimates made by two persons, or by the same person on different days, might vary perceptibly. More or less consciously, the criteria employed in these estimates have become gradually better defined, so that

in general the later estimates are probably more reliable than the earlier ones. One source of possible error, which was not at first appreciated, is the tendency of shoots resulting from recent regeneration to produce aberrant involucres. Since, in a culture that has for any reason become unhealthy, the proportion of regenerations is likely to be large, such a culture may, unless allowance is made for the disturbing factor, be credited with an undue proportion of tufted branches. For all these reasons, the proportions given, although estimated as carefully as possible, are to be considered only approximations.

The original culture of R35G, continued by means of occasional transplantations, was discarded on July 9, 1920. During the two years that it was in existence, it showed both tufted and typical branches, but no note was made of their relative proportions. A sub-clone (R35G1), started on February 28, 1919, from a tufted branch of R35G and still living, has been estimated at various times to contain two-thirds, three-fourths, four-fifths, and (this when the culture, as a result of recent transplantation, was relatively small), nine-tenths tufted branches—the other branches being either intermediate or predominantly or quite typical. Other sub-clones, started from parts of R35G1 and to be discussed more fully in another connection, have shown somewhat similar proportions of tufted and typical branches.

Figures 40 to 49, all drawn from plants of sub-clone R35G1 and one of its sub-clones, show something of the range of variations. Figure 42 represents a branch very similar in character to the parent plant of the clone; the involucres are crowded and most of them lobe-like, although the older part of the plant (at the lower left in the figure) bears a few involucres of approximately typical form. The branch shown in figure 41 is of the same general character, but the involucres are smaller, probably in consequence of conditions less favorable to their full development, and therefore not so crowded. However, on the majority of the branches that deviate so far from the typical character as to be classed as "tufted," most of the involucres, instead of being lobe-like, present such divergent forms as appear in figures 43 to 46. The irregularities that occur are so various that a full description would be out of the question. All the variations which have been described as occasionally appearing in a typical clone recur here; but often in very exaggerated degree. Frequently the involucres, as the figures show, are greatly inflated, with mouths irregularly enlarged, often lateral rather than terminal, and sometimes taking the form of long lateral slits. Involucral pouches and especially appendages, occasionally seen in a typical clone, are very common on the aberrant



FIGURES 40 to 49.—Plants and branches of a tufted ♀ clone (R35G). ×6.5.

involucres. Sometimes an appendage runs the greater part or all of the length of an involucre from base to apex, suggesting in such a case a seam along the line of junction of the margins of an originally lobe-like involucre. Such seam-like appendages, as well as shorter ones, not infrequently occur on the inner instead of the outer surface of an involucre.

As some of the figures indicate, the lateral lobes of a tufted branch often tend, like the involucres, to a marked irregularity of form. Since, however, the shape of lobes is a very variable character in typical clones, it has not been found practicable to use it as a distinctive character.

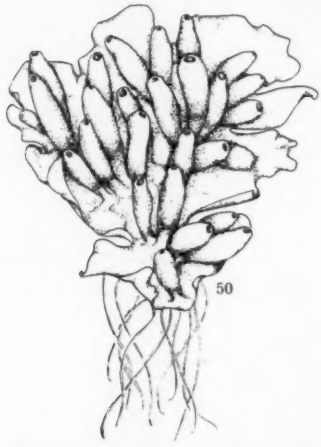


FIGURE 50.—Branch, typical in appearance, from a tufted ♀ clone (R35G). This branch was transplanted separately, on March 5, 1920, forming thus the starting-point of a sub-clone.

FIGURE 51.—Branch of the sub-clone derived from that shown in figure 50. On its older portion (lower right in the figure) the involucres are predominantly of typical form; the younger (upper left) portion shows the tufted character. ×6.5.

Figures 40 and 47 show the effect of exposure for some months to a saturated atmosphere, upon the habit of growth of a culture of this clone. These are comparable with figures 11 and 15, illustrating the effects of similar conditions upon a typical clone. It is evident that the appearance of the aberrancies characteristic of a tufted race is not affected by the conditions which so greatly modify the general proportions of the plant.

The branch shown in figure 43 bears two mature sporophytes. One sporophyte (at the upper right) is enclosed within an involucre of nearly typical form; the other (at the lower edge of the figure) is within an inflated involucre of very irregular shape, open on one side nearly or quite

to the base and thus exposing the capsule of the sporophyte, surrounded by its transparent calyptra. Near the center of figure 45, another capsule appears within an aberrant involucre.

Figure 49 represents a branch most of whose involucres are approximately typical, and figure 48 one which can in no way be distinguished from a branch of a typical clone.

The female sporeling shown in figure 21 resulted from a mating of clone R35G with a typical male clone (19.20). This sporeling gave rise to a tufted clone. A comparison with the typical female sporeling of figure 19 shows that not only do irregularities appear in the form of the earliest involucres, as is the case with typical sporelings, but that they continue to appear as the later involucres develop. The sporeling of figure 21 gave rise to a clone (20.304) which was estimated to contain, on two occasions, respectively, one-thirtieth and one-third tufted branches. While this seems to be the usual history of sporelings bearing the tufted tendency,— typical sporelings on the other hand ordinarily producing but few flaring or otherwise aberrant involucres,— there are too many variations to make it possible to determine with confidence the genotypic character of a very young plant.

THE CHARACTER OF A TUFTED MALE CLONE

Since tufted males have been derived only once directly from my original cultures, and since these have given rise to clones which grew slowly and remained small, the description here given of the tufted character as it is expressed in male plants is based upon the study of the gametophytic offspring of crosses between tufted females and typical males. One class among these offspring, as will appear more fully on later pages, is that of tufted males.

The tufted male clones consist of mixtures of tufted, typical, and intermediate branches, essentially as do the tufted female clones already described. As in the female clones, a tufted male plant may give rise to intermediate or typical branches.

Figure 39 shows the characteristic appearance of a tufted male clone (20.107), as contrasted with that of the typical clone represented in figure 38. Figures 52 to 58 are drawings of plants and branches of another tufted male clone (20.53). In figure 54 is shown a branch of substantially typical character; the other figures show various characteristic aberrancies in the forms of antheridial involucres.

In some cases the neck of an involucre, instead of being narrowly cylindrical, flares at the apex, the margin of the opening being incised,

toothed, or otherwise irregular. Sometimes the involucre is of about equal diameter throughout its length, a distinction between base and neck being absent. Not infrequently the neck is lacking; if the base is then of typical form, the antheridium is exposed at the top. Oftener, perhaps, the basal portion of such a neckless involucre is itself of aberrant form; being sometimes slit on one side or divided by vertical slits into two or more lobes; or expanded into the form of a cup, perhaps with a lobed or incised margin, or reduced to a saucer-like structure surrounding only the base of the antheridium and sometimes so small as to be invisible from above. In other cases the involucre is represented only by a lobe subtending the antheridium at one side. When the involucre is cup-like, saucer-like, or lobe-like, the antheridium is largely exposed (see especially figures 55, 57) as a glistening spherical body, green when young and becoming whitish at maturity. Evidently these variations in involucral form are comparable with those observed in the tufted females.

FIGURES 52 to 58.—Plants and branches of a tufted ♂ clone (20.53). ×8.4.

As the figures show, tufted males, like tufted females, display a decided tendency to variation in form and outline of thallus lobes; but here, again, the typical clones are sufficiently variable to make it impossible satisfactorily to separate tufted and typical races on the basis of lobe form.

In tufted males, as in the females, involucral appendages are more numerous than in typical races. The appendages have not been observed in as great numbers in the males as in the females; possibly, because of their small size, they are more likely in the males to be concealed from view or to be overlooked.

FREQUENCY OF TUFTED RACES

The first material of *Sphaerocarpos Donnellii* received was from the NEW YORK BOTANICAL GARDEN, on January 13, 1916. This had originated with plants obtained from Mr. SEVERIN RAPP, Sanford, Florida. The material when received bore numerous mature and immature sporophytes; the spore tetrads from a number of sporophytes were sown. From the cultures to which the germinated spores gave rise, three tufted female clones have been isolated, these coming from the spores from two sporophytes.

On February 4, 1916, living plants received directly from Mr. RAPP were divided into sixteen cultures, of which two furnished tufted females.

Of eight cultures started with plants received from Mr. RAPP on March 10, 1916, none was observed to contain certainly tufted plants.

Of ten cultures started with material received from Mr. RAPP on January 21, 1918, one contained tufted female plants.

Of fifteen cultures of material received from Mr. RAPP on February 23, 1918, four (one of which was R35) furnished tufted females.

Of six cultures from material, likewise from Mr. RAPP, received April 10, 1919, three supplied tufted female plants.

Material received from Mr. RAPP on March 20, 1918, contained numerous mature sporophytes. These plants were broken up and scattered upon the soil in seven pots. The cultures resulting from the germination of the spores (and in part, probably, from the regeneration of fragments of the old gametophytes) were not observed to contain tufted females. One of these cultures, however, contained some male plants that seemed to be tufted, and from two of these plants clones definitely showing the tufted character have been derived. These are the only certainly tufted male plants that have been found in, or derived directly from, any of the original cultures.

Among the descendants of plants of *S. Donnellii* received through the PLANT STUDY COMPANY on February 16, 1916, from Miami, Florida, no tufted races have been found. Nor have any comparable races been found in material of *S. texanus* received at different times from various sources.

The comparatively frequent occurrence of tufted races in the original cultures, and their genetic behavior as shown by the experiments reported in the present paper, indicate that strains of this genotypic constitution are regular, and probably not infrequent, constituents of the racial complex known as *Sphaerocarpos Donnellii*, at least as the species occurs about Sanford. The apparent rarity of tufted male strains is probably a con-

sequence of the lesser viability of male plants, as a result of which males of any type are comparatively rare in mixed cultures which have been grown for any length of time under greenhouse conditions. If tufted females occur in nature, tufted males must be proportionally about equally abundant, since, as will appear, tufted females cross readily with typical males, and tufted males appear among their offspring. Whether "tufted" strains display in nature the same characteristics that distinguish them in culture is a question to be settled by field study.

Miss HAYNES (1910, plate 29, figure 1), in her figure of a female plant of *S. Donnellii* (apparently drawn from dried material), shows variations in involucral form which suggest some of those here described. The plant drawn by her, however, would not, taken by itself, be classed as tufted.

C. and R. DOUIN (1917) have described and figured variations in the form of archegonial involucres of Sphaerocarpos, some having large mouths, others being longitudinally slit and thus resembling dorsal lobes. It is not clear whether these observations refer to *S. terrestris* (=*S. Michelii* Bell.) or to *S. californicus* (=*S. texanus* Aust.); but it is evident that aberrant conditions, suggesting those characteristic of the tufted character as here described for *S. Donnellii* have appeared in their cultures of one or both of these other species.

INTRACLONAL INHERITANCE OF THE TUFTED CHARACTER

The development of clones from isolated tufted plants, as already described, shows that a tufted plant (of the races I have studied), however aberrant its character, is capable of giving rise to branches and plants of strictly typical appearance. The observation of cultures so produced also shows that a plant of typical form belonging to such a clone is able, conversely, to produce tufted branches and plants. In order to test more fully the latter possibility, eight branches of strictly typical form from sub-clone R35G1, mainly from typical "patches" of the culture, were selected and transplanted to separate pots of soil. From each of these plants, in the course of a few months, a sub-clone of some size developed. Each of these sub-clones, like the parent clone, included a mixture of typical and tufted branches. Figures 48 and 50 are of two of the plants, typical in appearance, which thus gave rise to tufted sub-clones. In figure 51 appears a tufted branch of the sub-clone developed from the parent plant shown in figure 50. This parent plant was transplanted on March 20, 1920, and figure 51 was drawn on April 30. The youngest (upper left) part of the branch shown in figure 51 is of strictly tufted form;

the older part (which may include a portion of the original plant) is substantially typical. One of these sub-clones (R35G1a) was estimated at different dates to include about one-third, one-fourth, and one-half tufted branches; R35G1b, from which figures 49 and 50 were drawn, was similarly classed as one-half and two-thirds tufted. The other sub-clones were discarded as soon as it became evident that they were composed, like the parent clone, of mixtures of tufted and typical plants.

As a further test of the transmission of the tufted tendency through plants of typical appearance, twelve involucres of typical form, growing on branches strictly typical in appearance, were removed from culture R35G1b, and each was placed on the surface of the soil in a separate pot. RICKETT (1920 b) has shown that involucres, so treated, freely regenerate new thalli. Of the twelve involucres thus isolated, eight gave rise by regeneration to new sub-clones, each of which showed a mixture of tufted and typical branches. Seven of the eight sub-clones were grown until they had produced cultures of from one to three inches in diameter. Estimates of the proportions of tufted branches in these sub-clones were as follows: one-fourth; one-half; one-eighth to one-tenth, one-fifth, one-third; two-thirds, one-half; one-eighth, two-thirds; two-fifths; two tufted branches.

These experiments seem to demonstrate that the tufted potentiality—the ability, that is, to produce structures, especially involucres, of very aberrant form, is a character strictly fixed in the clone in question. The appearance within the clone of plants of intermediate and of typical character is thus apparently not an indication of what may be called, in a genetic sense, reversion or mutation. The tufted potentiality is inherent in the constitution of every cell of every plant in the tufted clone, and is passed on by each cell to its descendants. The numerous other tufted clones that have been isolated evidently agree in this respect with the one described. Since typical and tufted clones grown side by side for years, and subjected to the same wide range of environmental conditions, have throughout their history behaved entirely differently, it is clear that they are fundamentally and heritably different.

Although the genotypic basis of the tufted character is thus shown to be carried by the cells of a plant of typical form belonging to a tufted clone, the observations do not exclude the possibility that the formation of such a typical branch may involve a heritable change of the nature of a *weakening* of the tufted tendency. The admission of such a possibility would imply that a sub-clone derived from such a typical branch might show a less proportion of tuftedness than the parent clone. This possibility

is suggested by the proportion of tuftedness displayed, as shown above, by some of the sub-clones derived from typical branches or from typical involucres. However, since the proportion of tuftedness of the parent clone has been found to vary considerably from time to time, the smaller proportions observed in some of the sub-clones are far from conclusive. Evidently the possibility in question can be satisfactorily tested only by repeated selection, from a tufted clone and its descendants, of parts showing extreme tuftedness on the one hand and a strictly typical character on the other. If there should prove to be heritable differences, however small, between the typical and the tufted plants of the same clone, the selection would produce races approaching the typical condition and others, perhaps, showing higher degrees of tuftedness. Experiments of this nature are under way, but have not continued long enough to be decisive.

INHERITANCE THROUGH GAMETES AND SPORES

In each of the fertilization experiments to be reported, portions of a female and of a male clone were transplanted to the same pot, and, beginning either on the day of transplanting or some days later, were flooded several times, at intervals of one or more days, with boiled water. When sporophytes appeared and had matured, either the spores (or spore tetrads) were sown at once, or, more commonly, the female gametophytes bearing sporophytes were dried and kept until a convenient time for sowing the spores. Since all the activities of the species go on most vigorously during the cooler months, fertilizations have in general been made in late winter or spring, the sporophytes collected and preserved during the spring or early summer, and the spores sown in the following fall or winter. For how much longer time than this the spores may retain their viability has not been determined. The date given for the fertilization in each case is that of the first flooding of the pot containing male and female gametophytes.

In the cases of various matings involving tufted and other characters, attempts have been made, by sowing isolated tetrads, to obtain information as to the characters of the four gametophytes developed from the respective spores of a tetrad. The proportion of germination from such isolated tetrads was too small in nearly all my earlier experiments to give information of much value on this point. During the past two years (1922-1924) experiments of this nature have been successful on a large enough scale to promise definite results. But in the work reported in the present paper, a much larger proportion of germinations has been secured

by sowing the tetrads or spores of a capsule broadcast in a drop of sterile water upon the surface of the soil in a pot. Such results as were obtained by the other method are noted in connection with the respective experiments. When gametophytes appear after the sowing of the spores and are large enough to be handled fairly readily, they are isolated by means of needles under a binocular microscope and each is transplanted to a separate pot. In the transfer of a young gametophyte, especially if it is one of a group resulting from a broadcast sowing, there is always the possibility of transferring with it an ungerminated spore or another sporeling so small as to escape observation. This possibility has been guarded against by observing the developing clones from time to time with the aid of a binocular microscope, and by removing any part of the culture whose appearance suggested a separate origin. Rarely, when the part in question was certainly distinct,—if, for example, it was of opposite sex or differed decidedly in vegetative structure,—it has been transplanted and has served as the starting-point of a separate clone which is counted among the offspring of the mating in question.

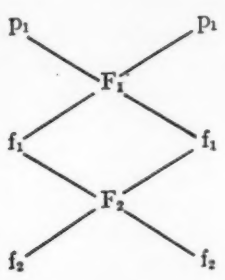
In case the gametophytes are allowed to grow, before being isolated, until the possibility of further germinations is substantially eliminated, there is the danger that two separated portions of a single plant may be isolated and counted as two. To avoid this possibility, in the few cases in which gametophytes were left for a relatively long time before transplanting, portions of plants have been selected here and there in the mixed culture, the remaining ones being discarded.

Each of these possible sources of error, together with the failure of some of the spores to germinate and with the death of some of the sporelings before they are isolated, may affect the apparent proportions of different classes among the offspring. If large enough numbers of plants are dealt with, there is little likelihood that any class of offspring will fail of observation.

The characteristics of the developing clones have been studied from time to time under the binocular microscope. Except for a few that died at an earlier stage, the female clones have in general been grown at least until they covered one-half or more of the soil in a three-inch pot. The males, growing more slowly, and often kept alive with more difficulty, have in general furnished clones filling a sixth to a half of such a pot.

In the description of the results of these matings, an extension of the ordinary Mendelian symbolism is necessary. The symbols P_1, F_1, F_2, etc., as used in work whether with plants or with animals, refer to diploid generations. In the study of gametophytic characters, it is convenient

to indicate the gametophytic (haploid) generations concerned by the small letters p_1, f_1, f_2, etc., retaining the use of capitals for the sporophytic (diploid) generations. The series of generations of Sphaerocarpos—or of any plant with an alternation of generations—may thus be represented, beginning with the parental gametophytes, by a scheme similar to the following:



(gametophyte)
(sporophyte)
(gametophyte)
(sporophyte)
(gametophyte)
etc.

The first fertilizations secured in the study here reported were of tufted females by typical males. It soon appeared, however, that the results of these crosses could be satisfactorily interpreted only in the light of a study of the offspring resulting from a fertilization of a typical female by a typical male. The results of such a mating will, therefore, be first described, although this was made later than some of the crosses. In general, the results of the experiments described in the following pages are not arranged in chronological order.

Typical ♀ × *typical* ♂

Mating 1. 19.24 × 19.27

Fertilized June 23, 1920. Female plants with mature sporophytes dried August 6. Spore tetrads sown November 1. Sporelings transplanted to separate pots February 1 to April 8, 1921.

The spore tetrads from two capsules (referred to as capsules A and B) were sown, respectively, upon the soil of two pots. The characters of the resultant clones (of the f_1 generation) are shown in table 1.

From table 1 it appears that, of 59 female clones of the f_1 generation whose character was determined, 41 (classed as "typical" in the table) showed a range of variation not noticeably greater than that of the ancestral clone; and that, of 33 male clones of the same generation, none showed a noticeably greater range of variation than did the ancestral male clone. There seems to be no important difference in these respects

TABLE 1

(p_1) *19.24* (♀ *typical*)×*19.27* (♂ *typical*).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	DIED YOUNG	TOTAL
Capsule A (F_1)				
f_1♀'s	23	12	3	38
f_1♂'s	21	0	9	30
Total	44	12	12	68
Capsule B (F_1)				
f_1♀'s	18	6	2	26
f_1♂'s	12	0	3	15
Total	30	6	5	41
Total f_1♀'s	41	18	5	64
Total f_1♂'s	33	0	12	45
Grand total (f_1)	74	18	17	109

between the groups of clones derived, respectively, from the spores produced in the two capsules (representing as many sporophytes of the F_1 generation). The clones noted as having "died young" were too small at the time of their death for their character to be conclusively determined; such observations as were made on these clones indicated that they were of substantially typical form.

The 18 female clones classed as "perhaps atypical" displayed no aberrant structures or characters except those especially affecting the size and form of involucres, already described as occurring in the p_1 clone 19.24. The sole basis on which these f_1 clones could be separated was the fact that at some time or times in its history each of them seemed to possess a larger proportion of variant involucres than did the p_1 female clone. At other times, however, each f_1 female clone showed no larger proportion of variations than did the p_1 female. Of these 18 f_1 female clones, 3 showed, at one or more times, from one to three branches each, the number of aberrant involucres on which was sufficient to justify a description of these branches as approaching the tufted form. At other times, however, each of these three clones possessed no such branches and showed no greater proportion of variations than did the p_1 female. At no time was a strictly tufted branch observed in any clone of this series; nor did any of the variant involucres approach the extremely aberrant conditions found in tufted races.

As between the 18 female clones thus tentatively classed as "possibly atypical," taking each at the time that it showed the greatest amount of variation in involucral form, there were differences in the proportion of aberrancies displayed by each. Similar proportional differences appeared among those female (as well as male) clones that are classed as "typical." While none of the clones in the "typical" group was observed to show a greater proportion of variation than was at some time displayed by the p_1 clone of the same sex, there were some which seemed, so far as observation showed, to display *less* variation in involucral form than did the p_1 clone. Discrimination in this respect being so largely a matter of individual judgment, as well as so much complicated by differences in the condition of the clones at different times, it was not found feasible to separate a class of offspring "more typical" or "less aberrant" than the parental form. Indeed, the distinction between the classes of "typical" and "possibly atypical" offspring is itself necessarily tentative.

Particular notice was taken of the presence or absence of involucral appendages, because this is a character in respect of which some clones vary greatly, and because also of the occurrence of numerous appendages in the tufted races. In general, the f_1 clones here considered did not differ markedly from the p_1 clones, in which, as already noted, these appendages occur infrequently. In the observations, made at various times, of the f_1 clones, often no appendages were observed in a particular clone; about as frequently, 1, 2 or 3 were found in a culture; less often, appendages were noted as "few," "rare," "several," or "occasional"; and much less often as "fairly frequent" or "not infrequent." A tendency was often noted for the appendages to be localized on particular branches; sometimes all the appendages observed in a culture were borne on the involucres of a single branch. In the latter case, two or even three appendages were occasionally borne by one involucre,—a condition otherwise very unusual. In one female and one male clone from spores of capsule A, and in 3 female clones from spores of capsule B, appendages were noted as "rather numerous" or "numerous." Only one of these, from a spore of capsule B, was among the female clones classified in table 1 as "possibly atypical." In each of these clones, at the times of other observations appendages were either few or entirely lacking. In 6 female and 8 male clones from spores of capsule A, and in 5 female and 3 male clones from spores of capsule B, no appendages were observed. It should, however, be added that 7 of the 11 last-mentioned female clones died after being subjected to only one detailed examination. While they were of sufficient size at the time of this examination to justify a classification as to their general

characters, it is quite possible that repeated examinations, such as were made of the great majority of the clones, would have shown at one time or another the presence of appendages. Note should be made, too, of the ease with which the variously situated, usually small, appendages may be overlooked,—especially, perhaps, in the male cultures. It can not be maintained that in any case every appendage in a culture was seen.

The net result of this experiment seems to be that, so far as observation has gone, the f_1 clones form a series which, phenotypically, ranges for the females from a condition more uniform to one showing somewhat greater variation in involucral form, including the presence of appendages, than that of the p_1 female; and, for the males, from a condition of greater uniformity to one of intraclonal variability at least equal to that characterizing the p_1 male. It is perhaps noteworthy that, among the f_1 clones, as among the individual plants of the p_1 clones, the males seem to show less variability than do the females.

This result may be explained genetically in at least two ways.

(a) It may be that all the f_1 clones of either sex are genotypically alike, and that the observed differences between them are purely phenotypic. That the range of these differences is greater than that observed within the p_1 clones may be explainable by the greater number of f_1 cultures under observation, and the consequently greater variety of conditions to which they were necessarily subject.

(b) It is also possible to suppose that the phenotypic differences observed in the f_1 generation have in part a genotypic basis, being to this extent due, in Mendelian terminology, to varied combinations of factors supplied by the p_1 generation. This involves the assumption that at least one of the p_1 clones possesses factors which, under some conditions, tend to bring about the variations observed, the two parental clones differing with respect to some or all of these factors. It must be remembered that there can be no question of dominance or recessiveness so far as the haploid generations here considered are concerned, and that therefore none of the characters of an f_1 plant can be explained as due to the action of recessive factors inherited from a p_1 ancestor. But meiosis in the F_1 sporophyte offers the possibility of new combinations of factors either through new groupings of the parental chromosomes or through crossing over, and, in consequence, of the appearance in some f_1's of more, and in some of less, aberrancy-producing factors than were present in either of the p_1 ancestors.

These alternative hypotheses may be tested by matings of the apparently more variable and of the apparently more uniform among the f_1 clones.

So difficult is it, however, to place the necessary distinctions upon a strictly objective and quantitative basis that the attempt to make such a test seems at present not very hopeful. Should the second hypothesis be sustained, it may be expected that one result of such further matings will

TABLE 2

(p_1) *R35G1* (♀ *tufted*) × *19.27* (♂ *typical*).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	CLASS 3 LESS THAN 10 PERCENT TUFTED	CLASS 4 10-50 PERCENT TUFTED	CLASS 5 50 PERCENT OR MORE TUFTED	DIED YOUNG	TOTAL
Capsule A (F_1)							
f_1♀'s	7	3	11	4	2	1	28
f_1♂'s	4	1	0	0	0	0	5
Total	11	4	11	4	2	1	33
Capsule B (F_1)							
f_1♀'s	5	1	1	0	1	0	8
f_1♂'s	0	0	0	0	0	0	0
Total	5	1	1	0	1	0	8
Capsule C (F_1)							
f_1♀'s	1	0	0	1	0	1	3
f_1♂'s	1	0	0	0	0	1	2
Total	2	0	0	1	0	2	5
Total f_1♀'s	13	4	12	5	3	2	39
Total f_1♂'s	5	1	0	0	0	1	7
Grand total (f_1)	18	5	12	5	3	3	46

Summary

	NON-TUFTED (CLASSES 1, 2)	TUFTED (CLASSES 3 to 5)	UNCERTAIN	TOTAL
f_1♀'s	17	20	2	39
f_1♂'s	6	0	1	7
Total	23	20	3	46

Died before sex or character was determined: capsule A, 3; capsule B, 3; total, 6.

be to secure clones that are more uniform than the "typical" clones selected as the starting-point in this work.

Tufted ♀ × *typical* ♂

Mating 2. R35G1 × 19.27

Fertilized January 24, 1921. Female plants with mature sporophytes dried April 12. Tetrads sown October 25. Sporelings transplanted January 7 to March 17, 1922.

This mating was one between the tufted female clone and the typical male clone already fully described. Clones of the f_1 generation were obtained from spores of three capsules. The classification of these clones is shown in table 2.

In table 2, classes 1 and 2 ("typical" and "possibly atypical") include clones of substantially the same characters as those similarly classified in table 1. Since two similar classes of offspring resulted from the mating of a typical female and a typical male (table 1), it seems reasonable to assume that the clones thus classed inherited no trace of the tufted character. Classes 3, 4 and 5 in the table include all the clones which at any time were observed to possess strictly tufted branches. Among the offspring of the present mating was one male clone showing sufficient variation to warrant its being placed in class 2, which class was not represented among the male offspring of mating 1. This male clone has always been small, never covering more than one-twentieth of the surface of the soil in a 3-inch pot. It is possible that the considerable proportion of aberrancies in involucral form which it has shown has been in part the result of its not very healthy condition, and that, had it grown more vigorously, it would have appeared strictly typical.

As shown in table 2, the number of male f_1 clones obtained was small (7 out of 46), and none of them was observed to be tufted. The absence of tufted male clones is probably, in view of the small number of males, not significant, since, as will be seen, other similar crosses yielded tufted males. The relative deficiency in numbers of male offspring obtained from this and from most of the other crosses to be described was undoubtedly largely, and probably entirely, due to the greater mortality of the male sporelings, both before and after their isolation. Thus, it is probable that most of the sporelings that died after isolation and before their sex was determined (6 in the present case) were males.

The tufted female clones are classed in the table according to the proportion of tufted branches possessed by them. Since the proportion of

tuftedness in each clone was estimated by the method already described, on two or (in most cases) more occasions separated by considerable intervals of time, it was necessary to average the results of different observations. This was done in such a way, taking into account the size of the culture at the time of each observation, as to determine the percentage that the total number of tufted branches constituted of the sum of all the branches of the clone at the time of the various observations. For example, the observations made on one female clone (22.85) were as follows:

On September 29, 1922, when the clone covered about one-seventh of the surface of a 3-inch pot, about two-thirds of its branches were tufted; on January 30, 1923, covering one-eighth of the surface, it had five-sixths tufted branches; on June 9, 1923, covering one-half of the surface, two-fifths of the branches were tufted. A relatively simple calculation shows that, on the basis of these estimates, 52 percent of all the branches present on these three occasions were tufted. This clone is therefore included in class 5 (50 percent or more tufted).

Since both the size of the culture and the proportion of tufted branches are estimated, and since the estimate of the proportion of tuftedness offers difficulties already alluded to, the percentage obtained in such a calculation might be materially modified if a larger number of observations of each clone had been made. However, as a general rule the different estimates for each clone were sufficiently similar to indicate that the percentages arrived at represent a really approximate statement of the character the clone has displayed during the time it has been grown. This being the case, the division of the tufted clones into a limited number of classes (as in table 2: less than 10 percent, 10 to 50 percent, and 50 percent or more tufted) should give a fairly reliable notion of the distribution of the degrees of the appearance of the tufted character. Obviously the number of classes thus to be made is an arbitrary one.

Among the clones referred to class 3 (less than 10 percent tufted) were 3, in each of which but one tufted branch was seen on one occasion, and 2, in each of which two tufted branches were seen on one occasion; at the times of other observations, each of these 5 clones appeared to be substantially typical. Evidently some of the clones now referred to classes 1 and 2 might at some time, if more observations had been made, likewise have shown one or a few tufted branches each, and so have been transferred to class 3. Also, since the difference between strictly tufted and intermediate branches is not a sharp one, another observer might have referred to class 3 a few of the clones here referred to class 2. It is also possible that a larger number of observations would have resulted in transfers in one direction or the other as between classes 3 and 4, or 4 and 5.

In a very general way, the proportion of aberrancies in involucral form on the non-tufted branches of a tufted clone is proportional to the degree of tuftedness as measured by the relative number of strictly tufted branches. Thus a clone which contains more than, say, 40 percent of tufted branches is likely to have a large proportion of other branches that may be classed as intermediate because of the number of atypical involucres that they bear. On the other hand, a clone with less than 10 percent of tufted branches ordinarily shows few, if any, more irregularities on its other branches than would be found on the branches of a typical clone. To this rule, however, there are fairly numerous exceptions.

Some notes were made of the relative abundance of involucral appendages in the clones of this family. But, since a much more thorough study was made of this point in another family from a similar cross, to be described below, and since there seems to be no material difference in this respect as between different families of similar origin, the matter will not be discussed here.

The clones now under discussion, like those produced from the mating typical × typical, could be arranged in a series, ranging in the present case from typical and very uniform (perhaps more uniform than the typical p_1 ancestor) to the extreme condition of tuftedness observed, which in this case was 73.1 percent (in a female clone). It may be noted that the percentage of tuftedness in the p_1 female clone, based upon four estimates, was 74.5,—a percentage substantially equalled by one f_1 clone. Thus this series of f_1 offspring, arranged according to their phenotypic characters, can be interpreted as ranging from a uniformity equal to, or perhaps greater than, that of the typical ancestor, to a tuftedness equal to that characteristic of the tufted ancestor.

It may well be questioned, in view of the intraclonal fluctuations in the expression of the tufted character, whether in such a series we are really dealing with more than two types of offspring—the non-tufted or substantially typical (classes 1 and 2), and the tufted (classes 3 to 5). The summary given at the conclusion of table 2 shows that, grouped in this way, the tufted and non-tufted female clones are approximately equal in number. This condition could be explained genetically by assuming a single factor for tuftedness in the p_1 female, allelomorphic (in the F_1) with the absence of such a factor or with a factor for uniformity.

On the other hand, the study of these clones, and of others derived from crosses to be reported later, has given a strong impression that the differences in degree of tuftedness represent in some measure fixed genotypic differences. With occasional marked exceptions, a clone once classed

(when of sufficient size to be fairly judged) as typical, was either typical or showed a very small proportion of aberrancies at all other times; one classed as showing a small proportion of aberrancies always showed a small proportion (or none); and one classed as largely tufted always showed a considerable proportion of tuftedness. A few illustrations taken at random will illustrate this point.

Clone 22.86 was classed on different occasions as three-fifths, two-fifths, and one-fourth tufted; clone 22.87, as one-tenth tufted, and (5 months later) as having only one tufted branch; clone 22.89, as typical, typical; clone 22.98, as possibly atypical, typical; clone 22.220, as possibly atypical (when small), typical, typical, typical; clone 22.241, as typical, typical, typical, typical; and clone 22.242, as typical (when small), one-third tufted, two-thirds tufted, one-sixth tufted.

In view of this tendency toward a measurable degree of persistence of distinctive characters, in cultures grown side by side under conditions as nearly identical as possible, it seems natural to conclude that there are fixed genotypic differences which are indicated, though of course not adequately measured, by such a classification as is used in table 2.

If this conclusion is valid, an adequate factorial hypothesis would involve the assumption of a difference of two or more factors between the tufted and the typical p_1 ancestors; the factors in question being concerned in the development or non-development of the aberrancies characterizing a tufted race. On this assumption, it would be expected that matings of largely tufted × largely tufted, and slightly tufted × slightly tufted would yield noticeably different results. Clones derived from such matings are now under cultivation.

Mating 3. R35G1×19.20

Fertilized January 17, 1920. Tetrads sown March 27. Sporelings transplanted December 9, 1920 to January 26, 1921.

The same tufted female clone was used in this mating as in mating 2. The male clone used as the parent was of the same ancestry as the typical male clone (19.27) already described, which was employed in matings 1 and 2. The spore from which it arose came, however, from a different capsule,—that is, the sporophytic parents of clones 19.27 and 19.20 were different individuals. This male clone has shown no marked differences from clone 19.27, having at no time been observed to produce branches with a larger proportion of variant involucres. It has therefore at all times been classed as typical.

Spores were sown from only one capsule resulting from this mating. The characteristics of the clones developed from these spores are shown in table 3.

The gametophytic offspring from this cross, the sex of all of which was determined, show, differently from the results of most of the matings, a preponderance of males. Among the males was the tufted class, which did not appear among the offspring of mating 2. In both sexes, in the family here considered, there is a marked preponderance of tufted over typical

TABLE 3

(p_1) *R35G1* (♀ *tufted*) × *19.20* (♂ *typical*).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	CLASS 3 LESS THAN 10 PERCENT TUFTED	CLASS 4 10 TO 50 PERCENT TUFTED	CLASS 5 50 PERCENT OR MORE TUFTED	TOTAL
f_1♀'s	3	0	6	3	4	16
f_1♂'s	9	0	3	8	2	22
Total	12	0	9	11	6	38

Summary

	NON-TUFTED (CLASSES 1, 2)	TUFTED (CLASSES 3 to 5)	TOTAL
f_1♀'s	3	13	16
f_1♂'s	9	13	22
Total	12	26	38

clones, and the numbers of individuals in both sexes showing the larger proportions of tuftedness (classes 4 and 5) are larger than among the offspring of mating 2. In view of the relatively small numbers, it may be questioned whether the more marked tendency in this family toward the appearance of the aberrancies characteristic of tuftedness is significant; if significant, the hereditary differences must have been contributed by the p_1 male parent, since the female parent was the same. It is conceivable that the p_1 male may have possessed factors which, while by themselves producing no visible effect, contributed to an intensifying of the tufted character when joined with a factor or factors for tuftedness derived from the p_1 female.

It should be pointed out that the classification of four of the clones of this family is open to some question.

Clone 20.330 (♀) at the time of one observation was noted as having 2 tufted branches; on another occasion, 3 branches suggested an approach to the tufted condition; on three occasions, the clone appeared typical.

Clone 21.2 (♂) at one time showed 2 branches classed as tufted; at another time it was strictly typical.

Clone 21.5 (♀) showed at one time 2 branches classed as tufted; on another occasion it was quite typical.

The very few "tufted" branches in these cases might perhaps have been classed by another observer as not strictly tufted, the three clones thus being assigned to class 2 (possibly atypical) rather than to class 3 where they are placed in table 3, so reducing somewhat the apparent preponderance of the tufted over the non-tufted classes.

On the other hand, clone 21.3 (♂) died when very small. The few branches observed were typical, and the clone is thus classified; but, judging from experience in other cases, such a clone, had it grown to some size, might have shown some proportion of tuftedness.

As to all the other clones in this family, the classification is made with confidence in its approximate correctness. The extreme of tuftedness found was 72.9 percent in a male clone. This, again, is substantially the same as the degree of tuftedness of the p_1 female.

Mating 4. R35G×R42C1

Fertilized March 8, 1919. Tetrad sown June 7. Sporeling transplanted December 2.

The maternal clone in this mating was that of which a sub-clone was used in matings 2 and 3. The male plants used were separated from a mixed culture. They did not, therefore, necessarily represent a single clone. So far as observed, they were all of typical character.

This was among the earliest crosses attempted. 87 tetrads from two capsules were sown on soil in individual pots, and 16 tetrads from one of the same capsules were sown in sterilized Marchal's solution. Only one sporeling (19.30) was obtained,—from a spore germinating on soil. It is included here for the sake of completeness.

The sporeling in question was female. At first it was taken to be typical; but as the clone developed, tufted branches appeared. It was thus made evident that the enumeration of tufted and typical clones can be made with certainty only after each has grown to some size. The averaging

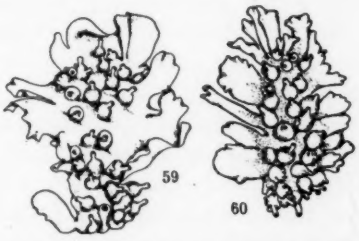
of later observations by the methods above described shows this clone to have possessed about 36.3 percent tufted branches. It would therefore fall within class 4 as defined in previous tabulations.

Mating 5. R35G×PSC2Bα3

Fertilized October 17, 1918. Tetrads sown February 18 and 22, 1919. Sporelings transplanted May 5.

This, also one of the earliest crosses attempted, likewise yielded meager results. The p_1 female parent was the same as in the previous mating. The p_1 male (or males) belonged to a race developed from two male sporelings, probably from the spores of a single tetrad, borne by a sporophyte resulting from flooding a mixed culture of the plants received from Miami, February 16, 1916. This culture was flooded immediately upon being potted and on several other occasions during the succeeding three months. On June 16, the tetrads from one capsule were sown on a pot of soil, and on March 10, 1917, various groups of sporelings were removed and those of each group (presumably from a single tetrad) were planted in a separate pot. One of these groups consisted of two male plants, from which developed the male race used in the present mating.

As already noted, no tufted plants of either sex have been found among the Miami material. The male race used in this mating has shown itself to be typical throughout its history, so far as the absence of tuftedness is concerned. It has, however, been characterized by a tendency to produce considerable numbers of involucral appendages. Two plants of the race showing something of this tendency are illustrated in figures 59 and 60. The appendages vary in number on different branches; some bear few or no appendaged involucres, whereas on certain branches (see the lower part of figure 60) appendages are so numerous as to give the involucres a quite unusual appearance.



FIGURES 59, 60.—Plants of a ♂ race (PSC2Bα3) showing an unusual proportion of involucral appendages. ×8.4.

Various methods were used in the attempt to germinate the spores borne by the sporophytes resulting from the mating now in question. Only two sporelings were obtained, the spores giving rise to them having been obtained from different capsules.

One of the two offspring clones is female. While still young, this clone showed itself plainly tufted. In the earlier observations the proportion of tuftedness was not noted. A calculation based upon four later observations shows 19.6 percent of tuftedness. The clone therefore falls in class 4 as previously defined.

The second clone is male. At first it was classed as typical, and no strictly tufted branches were observed until the clone had been growing for nineteen months. On December 4, 1920, one tufted branch was observed, and on May 30, 1921, one-tenth of the branches were classed as tufted. On one later occasion the clone appeared strictly typical, and at the time of each of three later observations no tufted branches, but two approaching the tufted form, were seen. The clone, with an average of 1.2 percent tuftedness, is placed in class 3.

Neither of these clones showed at any time more than very rare appendages on involucres of typical or approximately typical form. It would appear, then, that they did not inherit the appendiculate character of the male p_1 ancestor.

Mating 6. R35F×19.21

Fertilized January 17, 1920. Tetrads sown April 2. Sporelings transplanted July 10, 1920 to February 1, 1921.

The female clone used in this mating originated in a tufted plant isolated from the same mixed culture from which R35G was obtained. Clone R35F has shown itself (average of three observations) about 57.4 percent tufted.

The male clone used was derived from a spore of a different tetrad but from the same capsule as that which gave rise to clone 19.20 (used in mating 3). This clone has been uniformly classified as typical, showing about the same proportion of variations in involucral form as do clones 19.20 and 19.27.

Sporelings were obtained from the spores of two capsules resulting from this mating. 29 tetrads from capsule A were sown separately, one to a pot. From these tetrads, 7 sporelings were obtained, 2 from one tetrad, 1 from each of five others. Of the two clones obtained from spores of a single tetrad, both were female; one was tufted (16.4 percent), the other died before its character was determined. The tetrads from capsule B were sown broadcast, and the sporelings were transplanted separately as previously described. The characteristics of the clones thus obtained from the spores of the two capsules are shown in table 4.

TABLE 4

(p_1) R35F (♀ tufted)×19.21 (♂ typical).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	CLASS 3 LESS THAN 10 PERCENT TUFTED	CLASS 4 10 TO 50 PERCENT TUFTED	CLASS 5 50 PERCENT OR MORE TUFTED	DIED YOUNG	TOTAL
Capsule A (F_1)							
f_1♀'s	0	0	0	1	0	2	3
f_1♂'s	1	0	0	0	1	0	2
Total	1	0	0	1	1	2	5
Capsule B (F_1)							
f_1♀'s	14	2	12	4	0	0	32
f_1♂'s	9	0	6	7	5	0	27
Total	23	2	18	11	5	0	59
Total f_1♀'s	14	2	12	5	0	2	35
Total f_1♂'s	10	0	6	7	6	0	29
Grand total (f_1)	24	2	18	12	6	2	64

Summary

	NON-TUFTED (CLASSES 1, 2)	TUFTED (CLASSES 3 to 5)	UNCERTAIN	TOTAL
f_1♀'s	16	17	2	35
f_1♂'s	10	19	0	29
Total	26	36	2	64

Died before sex or character was determined: capsule A, 2; capsule B, 4; total, 6.

The classification as to character in table 4 is based upon only two observations of the character of each clone; in one case (a male, 50 percent tufted), upon only one observation. The information, therefore, is less full than in the cases of most of the clones in the other families reported. However, experience with other families more extensively studied indicates that the classification here given would not have been materially altered by additional observations. In the cases of one male and five female clones placed in class 3, one observation showed from one to three tufted branches; the other observation in each case showed the clone to

be typical. As in previous instances, a question might perhaps be raised as to the propriety of the classification of these clones.

The sexes are approximately equal in numbers in this family, such preponderance as there is being, as usual, in favor of the females. There is also, as in mating 3, a larger number of tufted (classes 3 to 5) than of non-tufted clones (classes 1, 2), and the males, although fewer in total number, preponderate over the females in the classes (4 and 5) of greater proportional tuftedness. Five male clones show a higher proportion of tuftedness (respectively, 60, 63.6, 75, 80, and 87.7 percent), than that which on the average characterized the tufted p_1 ancestor (57.4 percent).

Mating 7. R35.1B×19.4

Fertilized January 17, 1920. Tetrads sown April 2 and 3. Sporelings transplanted July 10 to August 21.

The female clone used in this mating was derived from a tufted plant taken from the same mixed culture as were R35F and R35G. It has shown an average tuftedness (in three observations) of 68.5 percent.

The male clone originated from a spore from the same capsule from which came the spores that were the starting-points of clones 19.24 (♀) and 19.27 (♂). It has uniformly shown the typical character.

From one capsule resulting from this cross, 19 spore tetrads were sown separately. Only one sporeling was obtained, and it died before its sex or character was determined.

From a second capsule, 18 tetrads were sown separately. From these, 7 sporelings were obtained, of which 3 died before sex or character was definitely determined. Of the clones developed from the survivors, two were typical females, one a female 17 percent tufted, and one a male 8 percent tufted. Three of these clones, the two typical females and the tufted male, came from spores of a single tetrad.

Mating 8. R27E×R27D

Fertilized March 8, 1919. Female plants with mature sporophytes dried May 30. Spores sown December 24, 1919 to January 10, 1920. Sporelings transplanted February 2 to April 4, 1920.

This was another of the earlier matings, but it gave more extensive results than others attempted at about the same time.

The female clone here used originated in a tufted plant received from Mr. RAPP, January 21, 1918. The clone has shown throughout its history a high degree of tuftedness. The earlier notes made as to its character

were merely to the effect that it appeared to be markedly tufted. Five later, more definite observations (ranging from three-fourths to nineteen-twentieths tufted) give an average of 86 percent of tuftedness.

This female clone is also characterized by the fact that sporophytes produced by the fertilization of its eggs have regularly borne spores that were separate at maturity, or were at most held together by fragments of fragile tetrad walls that broke readily when the spores were removed from the capsule. All sporophytes produced in my cultures of *Sphaerocarpos Donnellii*, except those borne by this clone and by some of its descendants, bear spores held together in tetrads by firm, resistant tetrad walls. When the separate spores were first observed in sporophytes borne by clone R27E, it was thought that their separation might have resulted from some exceptional external conditions. This first observation was noted in a previous paper (ALLEN 1919, p. 294, footnote). But fertilizations at various times by male races of diverse origin have shown that the production of separate spores is a uniform characteristic of the sporophytic offspring of this clone. A discussion of the inheritance of this character is reserved for a later paper.

The male race used in this mating originated in a group of plants transplanted from the same mixed culture as the single plant that gave rise to the female clone. The male race is therefore not certainly a clone, and its genetic purity is open to some question. However, during about one year that it was kept under observation, no tufted plants were seen in it, and the culture appeared uniformly typical.

The characters of the gametophytic offspring derived from this cross are shown in table 5. The spores of capsules A to E were sown broadcast; those of capsules F and G were sown singly in individual pots,—as the table shows, with scanty results.

This family includes the largest number of f_1 clones thus far obtained from a single mating of tufted and typical parents. It has also been under observation longer than any other family of considerable size. The earlier observations, to be sure, were based upon imperfectly developed criteria, and in connection with most of them no attempt was made to determine the proportion of tuftedness in individual clones. In calculating percentages of tuftedness, therefore, the earlier notes are of little value. Nevertheless, for each of those clones that have remained alive for three years or more, the number of separate estimates upon which the determination of character rests ranges from five to much larger numbers. Unfortunately, some of the clones, especially a considerable proportion of the males, died when comparatively young, and the determinations of

TABLE 5

(f_1) *R27E* (♀ *tufted*) × *R27D* (♂ *typical*).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	CLASS 3 LESS THAN 10 PERCENT TUFTED	CLASS 4 10 TO 50 PERCENT TUFTED	CLASS 5 50 PERCENT OR MORE TUFTED	DIED YOUNG	TOTAL
Capsule A (F_1)							
f_1♀'s	6	4	10	8	6	2	36
f_1♂'s	22	0	2	0	8	1	33
Total	28	4	12	8	14	3	69
Capsule B (F_1)							
f_1♀'s	0	1	1	2	0	0	4
f_1♂'s	5	0	0	0	0	1	6
Total	5	1	1	2	0	1	10
Capsule C (F_1)							
f_1♀'s	2	3	1	0	5	1	12
f_1♂'s	3	1	0	2	3	4	13
Total	5	4	1	2	8	5	25
Capsule D (F_1)							
f_1♀'s	2	0	2	1	1	0	6
f_1♂'s	3	0	0	0	1	1	5
Total	5	0	2	1	2	1	11
Capsule E (F_1)							
f_1♀'s	0	1	5	4	4	0	14
f_1♂'s	4	0	2	0	0	3	9
Total	4	1	7	4	4	3	23
Capsule F (F_1)							
f_1♀'s	0	0	0	0	0	0	0
f_1♂'s	1	0	1	0	0	0	2
Total	1	0	1	0	0	0	2
Capsule G (F_1)							
f_1♀'s	0	0	0	1	0	0	1
f_1♂'s	0	0	0	0	0	1	1
Total	0	0	0	1	0	1	2
Total f_1♀'s	10	9	19	16	16	3	73
Total f_1♂'s	38	1	5	2	12	11	69
Grand total	48	10	24	18	28	14	142

TABLE 5 (continued)
Summary

	NON-TUFTED (CLASSES 1, 2)	TUFTED (CLASSES 3 to 5)	UNCERTAIN	TOTAL
f_1♀'s	19	51	3	73
f_1♂'s	39	19	11	69
Total	58	70	14	142

Died before sex or character was determined: capsule C, 1; capsule E, 1; capsule G, 1; total, 3.

their characters are much less satisfactory. Thus, of 38 male clones classed as typical, 28 remained small and died within the first year. There can be little doubt that, had these 28 clones survived for a longer time, some, perhaps the majority, of them would have shown some degree of tuftedness; in other words, the number of males referred to class 1 in table 5 is probably too large, and the numbers referred to classes 3 and 4 are probably too small.

On the other hand, there are 2 male and 6 female clones classified as slightly tufted (class 3), in each of which, on one occasion, one or a very few tufted branches were observed, whereas at the times of other observations they appeared strictly typical. As previously noted, it is not easy to draw a sharp line between classes 2 and 3, and it is quite possible that another observer would have referred these clones, or some of them, to class 2. In these cases, then, the error, if any, is in the direction of increasing the apparent number of tufted clones. On the whole, there is little doubt that a tendency exists, in the classification of this as well as of the other families here considered, toward a net error in favor of the non-tufted classes. It is worthy of note, therefore, that the totals for this family (summary, table 5) show a preponderance of the tufted classes, considered together, over the non-tufted classes; and that this preponderance becomes much more marked when only the female clones, whose classification is more reliable, are considered.

Among the male clones growing from the spores of capsule A is one (20.45), referred in the tabulation to class 5, which is possibly a new form. This clone has been from the start markedly tufted, showing all the irregularities of the tufted races and only rarely producing a branch that is typical or approximately typical. It has been distinguished by a luxuriance of vegetative growth, shown especially in the size of the lateral lobes, and by a relative scarcity of antheridia and involucres. Whether this clone is genotypically distinct from other tufted races is a question

to be settled by future experiment. It is the only instance suggestive of a mutation that has appeared in any of the cultures discussed in the present paper.

Of the clones included in class 5 (50 percent or more tufted), six, including three males and three females, show degrees of tuftedness somewhat higher than that of the p_1 female ancestor. The highest percentage of tuftedness has been shown by a male clone which has 92.9 percent as compared with the 86 percent characterizing the p_1 female.

Special attention has been paid to the occurrence of involucral appendages in this family. The frequent occurrence of these appendages, often in exaggerated form, has been described as characteristic of the variously aberrant involucres of the tufted branches. Involucres of typical or approximately typical form on the tufted branches also not infrequently bear appendages; and occasionally a branch, otherwise typical, bears several involucres with one, two, or three appendages each. These observations suggest the possibility that the occurrence of appendages may be an expression of the same tendency which in other branches manifests itself in the markedly atypical forms of involucres.

On the other hand, appendages, usually rare, occur on the involucres of many clones otherwise strictly typical; and occasionally they appear on two or more involucres of a particular branch or particular branches, when, so far as careful search shows, they are absent from all the other branches of the same clone. It is conceivable, therefore, that the production of involucral appendages may be brought about in two ways: both as a part of the expression of the "tufted" tendency, and as the expression of another and quite distinct tendency. Consonant with this notion is the occurrence of typical clones which have not been observed to bear appendages, and of others which are typical save for the appearance of appendages in considerable numbers.

A third possibility is perhaps not entirely out of the question. This is, that the production of involucral appendages in differing proportions represents some of the steps in a series of hereditary possibilities, other steps in which are shown by clones with markedly different degrees of tuftedness. Such a series of genotypically different forms, if such a series really exists, has at one extreme the clones presenting the closest approach to uniformity in involucral form, and at the other those with the highest percentages of tuftedness.

The recorded observations bearing on the appearance of involucral appendages in the female clones are more satisfactory than those made on the male clones—both because so many of the latter died at an early

age and because the appendages on the antheridial involucres are smaller and more easily overlooked. Table 6 gives a summary of the observations as to the proportions of appendages borne on otherwise typical involucres in the respective female clones of this family.

TABLE 6

	FREQUENCY OF APPENDAGES				TOTAL
	None	One to few	Occasional	Not infrequent	
Non-tufted clones	4	14	1	0	19
Tufted clones	15	20	7	9	51

The fact that no appendages were seen on the otherwise typical involucres of some clones is not conclusive evidence that none were at any time present; but it is certain that in these clones such appendages have at any rate been very rare.

The tabulation given above makes it clear that the distribution of appendages borne on involucres of typical form is not very different as between the tufted and the non-tufted clones, except for the class of tufted clones in which such appendages are noted as "not infrequent." In this class, and perhaps in the class with "occasional" appendages, it is probable that the appearance of the appendages on involucres of typical form is in part an expression of the tufted tendency inherited from the p_1 female. Further information on the inheritance of the capacity for appendage-production, as distinct from the tufted tendency, must be supplied by matings made with reference to this character alone.

Matings 9 and 10. R27E×19.27

Mating 9. Fertilized January 17, 1920. Spores sown April 1. Sporelings transplanted July 8, 1920 to January 27, 1921.

Mating 10. Fertilized January 24, 1921. Plants with mature sporophytes dried April 12. Spores sown October 25 to 31. Sporelings transplanted January 12 to April 25, 1922.

Two matings were made of these parents,—the female being the tufted clone used in mating 8, and the male the typical clone used in matings 1 and 2. The results are combined in table 7; the sporophyte-producing capsule A resulted from mating 9; capsules B, C and D were obtained from mating 10. In each case the spores (not united in tetrads) were sown broadcast.

TABLE 7

(f_1) *R27E* (♀ *tufted*) × *19.27* (♂ *typical*).

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	CLASS 3 LESS THAN 10 PERCENT TUFTED	CLASS 4 10-50 PERCENT TUFTED	CLASS 5 50 PERCENT OR MORE TUFTED	DIED YOUNG	TOTAL
Capsule A (F_1)							
f_1♀'s	4	5	11	2	4	4	30
f_1♂'s	6	2	1	5	4	1	19
Total	10	7	12	7	8	5	49
Capsule B (F_1)							
f_1♀'s	2	1	7	0	0	0	10
f_1♂'s	1	0	1	0	0	0	2
Total	3	1	8	0	0	0	12
Capsule C (F_1)							
f_1♀'s	0	1	0	0	0	1	2
f_1♂'s	0	0	0	0	0	1	1
Total	0	1	0	0	0	2	3
Total f_1♀'s	6	7	18	2	4	5	42
Total f_1♂'s	7	2	2	5	4	2	22
Grand total (f_1)	13	9	20	7	8	7	64

Summary

	NON-TUFTED (CLASSES 1, 2)	TUFTED (CLASSES 3 to 5)	UNCERTAIN	TOTAL
f_1♀'s	13	24	5	42
f_1♂'s	9	11	2	22
Total	22	35	7	64

Died before sex or character was determined: capsule A, 8; capsule D, (all) 3; total, 11.

It will be observed that in this family there is a preponderance in both sexes (marked in the female) in number of tufted as compared with non-tufted clones.

The general nature of the evidence as to the character of the f_1 clones in this family is substantially as in connection with the families previously discussed. The highest average tuftedness found (in a male clone) was 84 percent; the p_1 female averaged, as already noted, 86 percent.

Summary of matings 2 to 10

Table 8 summarizes the results of the crosses here reported (matings 2 to 10) between tufted females and typical males. When, as in the second part of the table, the f_1 offspring of these crosses are combined into two groups,—"non-tufted" and "tufted,"—the predominance of tufted over non-tufted females is striking (128 as compared with 70). The numbers of males in these two groups, on the other hand, are nearly equal, the non-tufted males being somewhat more numerous. This discrepancy between the distribution of males and that of females is mainly due, however, to the apparent predominance of non-tufted over tufted males among the offspring of mating 8. Reasons have been given for considering the classification of the male clones in this family,—and, to some extent, in other families,—unsatisfactory. If, as there is reason to suspect, a considerable proportion of the males classed as non-tufted would, had they survived long enough, have shown some degree of tuftedness, the distribution of males as non-tufted and tufted might approach that of females.

If such a correction were made, the tufted f_1 clones in each sex would considerably outnumber the non-tufted clones,—perhaps approximately in the proportion of two to one. The figures for the whole population, therefore, like those for the larger families considered separately, may possibly be harmonized with a hypothesis that the character of the p_1 female is due to the presence of two or more factors for tuftedness.

Something of the same sort is suggested by the distribution of the f_1 female clones according to degrees of tuftedness, as shown in the first part of table 8. Classes 1 and 2 (non-tufted) include 70 females; class 3 (less than 10 percent tufted) contains 67 females; classes 4 and 5 (showing larger proportions of tuftedness) contain together 61 females. These figures might be taken to indicate that the clones referred to in class 3 are, in general, those which have received only one factor for tuftedness as a result of the redistribution of the chromosomes in spore-formation; while classes 4 and 5 include, in the main, those which received two or more such factors. Here, again, the totals for the males do not support the assumption; for the males in class 3 (18) are fewer than those in either class 4 or class 5 (22 and 24, respectively). The correction suggested for the

f_1 males would again bring the figures for the males and for the females more nearly into harmony—since some proportion of those now placed in class 1 would be transferred to class 3. It is evident that the discussion of such points must be highly tentative until more extensive results are available.

TABLE 8

Summary of all matings of tufted ♀ × typical ♂.

	CLASS 1 TYPICAL	CLASS 2 POSSIBLY ATYPICAL	CLASS 3 LESS THAN 10 PERCENT TUFTED	CLASS 4 10 to 50 PERCENT TUFTED	CLASS 5 50 PERCENT OR MORE TUFTED	DIED YOUNG	TOTAL
Mating 2							
f_1♀'s	13	4	12	5	3	2	39
f_1♂'s	5	1	0	0	0	1	7
Mating 3							
f_1♀'s	3	0	6	3	4	0	16
f_1♂'s	9	0	3	8	2	0	22
Mating 4							
f_1♀'s	0	0	0	1	0	0	1
f_1♂'s	0	0	0	0	0	0	0
Mating 5							
f_1♀'s	0	0	0	1	0	0	1
f_1♂'s	0	0	1	0	0	0	1
Mating 6							
f_1♀'s	14	2	12	5	0	2	35
f_1♂'s	10	0	6	7	6	0	29
Mating 7							
f_1♀'s	2	0	0	1	0	0	3
f_1♂'s	0	0	1	0	0	0	1
Mating 8							
f_1♀'s	10	9	19	16	16	3	73
f_1♂'s	38	1	5	2	12	11	69
Matings 9, 10							
f_1♀'s	6	7	18	2	4	5	42
f_1♂'s	7	2	2	5	4	2	22
Total f_1♀'s	48	22	67	34	27	12	210
Total f_1♂'s	69	4	18	22	24	14	151
Grand total (f_1)	117	26	85	56	51	26	361

Died before sex or character was determined, 30

TABLE 8 (continued)

Summary

	NON-TUFTED (CLASSES 1, 2)	TUFTED (CLASSES 3 to 5)	UNCERTAIN	TOTAL
Mating 2				
f_1♀'s	17	20	2	39
f_1♂'s	6	0	1	7
Mating 3				
f_1♀'s	3	13	0	16
f_1♂'s	9	13	0	22
Mating 4				
f_1♀'s	0	1	0	1
f_1♂'s	0	0	0	0
Mating 5				
f_1♀'s	0	1	0	1
f_1♂'s	0	1	0	1
Mating 6				
f_1♀'s	16	17	2	35
f_1♂'s	10	19	0	29
Mating 7				
f_1♀'s	2	1	0	3
f_1♂'s	0	1	0	1
Mating 8				
f_1♀'s	19	51	3	73
f_1♂'s	39	19	11	69
Matings 9, 10				
f_1♀'s	13	24	5	42
f_1♂'s	9	11	2	22
Total f_1♀'s	70	128	12	210
Total f_1♂'s	73	64	14	151
Grand total (f_1)	143	192	26	361

Died before sex or character was determined, 30

Another question naturally arising is whether the different tufted female clones used as parents in the respective matings are alike in their genetic constitution. If, for example, one of the female clones thus used is to be considered as possessing two independent factors for tuftedness,

it is conceivable that another female clone which was mated with a typical male might possess one, or three, such factors.

In matings 2 to 5, inclusive, the female parent was R35G, or one of its sub-clones. The estimated percentage of tuftedness of this clone is 74.5. In mating 2, the maximum tuftedness among the f_1 clones was 73.1 percent; in mating 3, 72.9 percent. Matings 4 and 5 gave too few offspring to furnish significant data upon this point.

R35F, with 57.4 percent estimated tuftedness, was the female parent in mating 6. The maximum tuftedness among its f_1 offspring was 87.7 percent. It may be suspected that the percentage of tuftedness found for the female parent in this instance might have been higher had a larger number of observations been made.

R35.1B was the female parent in mating 7; but here the number of f_1 offspring was too small to give significant results.

The female parent in matings 8 to 10, inclusive, was R27E, with 86 percent tuftedness. In mating 8, the maximum percentage of tuftedness among the f_1 clones was 92.9; in matings 9 and 10 (in which the male parent was the same), the maximum percentage was 84. In comparing the offspring of R35G and R27E (those giving the largest total numbers of offspring), it appears that the latter parent, with the larger estimated proportion of tuftedness, produced individual offspring with a higher degree of expression of the same character. The difference is perhaps great enough to suggest a difference in the genetic constitution of these two parental clones with reference to the tufted character; but it is at any rate not conclusive.

If the proportions of non-tufted to tufted be considered, comparing the f_1 offspring of R 35G(matings 2 to 5) with those of R27E (matings 8 to 10), the results are as follows:

Female offspring of R35G: non-tufted 20, tufted 35; of R27E: non-tufted 32, tufted 75.

The larger proportion of tufted clones among the f_1 offspring of R27E may be significant; the argument is weakened, however, by the corresponding figures for the male offspring, which are:

Male offspring of R35G: non-tufted 15, tufted 14; of R27E: non-tufted 48, tufted 30.

The fact probably is, as already pointed out, that some of the male clones classed as non-tufted were really tufted; but how closely the necessary correction would make the distribution of the males agree with that of the females is questionable.

On the whole, the indication is fairly strong that the tufted parents used in the respective matings are genetically different, and that the

offspring of each mating fall into different classes with respect to their inheritance of the tufted tendency.

If the figures for the male and those for the female offspring were equally reliable, the difference between the sexes as to the distribution of tuftedness might be explained by assuming that at least one factor concerned in the production of this character is sex-linked. It must be remembered that, since the female gametophyte has but one X chromosome, which is transmitted through the sporophyte to the female offspring, a factor borne on the X chromosome would find expression (so far as the gametophytic generation is concerned) only in the females. Since, however, there is so much doubt as to the classification of the male clones in the families here discussed, speculation upon this possibility is at present useless.

Typical (f_1) ♀ × *typical* ♂

Mating 11. 20.124×19.27

Fertilized January 25, 1921. Female plants with mature sporophytes dried April 12. Spores (not in tetrads) sown broadcast October 25 to 31. Sporelings transplanted February 14 to April 25, 1922.

The results of one mating are to be reported in which the maternal clone was a typical f_1 offspring of mating 8 (R27E×R27D). The male clone with which this was mated was 19.27, the one selected for description as a typical male and used in matings 1, 2, 9 and 10.

Spores were sown from three capsules of sporophytes resulting from mating 11. The spores from capsule A gave rise to 5 clones, of which one died while very young; the spores from capsule B to 7 clones, of which one died while young; and the spores from capsule C to 9 clones, of which one died while young. Of the 18 f_1 clones which thus lived long enough to allow of a satisfactory determination of their sex and character, 14 were females, 4 males. All the male clones were typical; of the female clones, 10 were typical, 4 possibly atypical; none bore branches that were classed as tufted, although in one of the "possibly atypical" female clones one branch appeared which approached the tufted condition. Similar branches, it may be recalled, were occasionally borne by offspring of mating 1.

Thus, so far as can be judged from the relatively small numbers in this family, the typical f_1 clone descended from a tufted p_1 female produces the same classes of descendants as does a typical clone of typical ancestry (compare table 1). The typical f_1 clone of mixed parentage passes on to its descendants no tufted tendencies; that is, there is nothing correspond-

ing to the reappearance of a recessive character. This, of course, is what would be expected in dealing with the characters of the haploid generation.

SUMMARY

1. A clone (female or male) of *Sphaerocarpos Donnellii* manifests a wide range of variation in response to varied cultural conditions. In a clone selected as substantially typical for the species, the variation, under the conditions to which the cultures here described have been exposed, is not so great as to prevent the appearance of certain fairly definite and easily recognizable characters.

2. A considerable number of female clones and two male clones, showing what is here called the "tufted" character, have been isolated from material originally derived from Sanford, Florida. Tufted clones are marked especially by numerous and striking aberrancies in the forms of involucres. Such clones are extremely variable, in the sense that their respective branches show transitional conditions ranging from strictly typical to extremely aberrant.

3. A tufted clone is genotypically different from a typical clone, as shown by the fact that a sub-clone developed vegetatively from a branch of strictly typical appearance produced by a tufted clone, or from a typical involucre borne on such a branch, always produces very aberrant branches. A typical clone never produces such branches.

4. The gametophytic offspring of a typical female mated with a typical male include clones of both sexes, which, while they seem to show an interclonal variability somewhat greater than the intraclonal variability of either gametophytic ancestor, never display the tufted character.

5. The gametophytic offspring of a tufted female mated with a typical male include four classes: typical females, tufted females, typical males and tufted males.

6. Allowing for errors in classification, it appears that the offspring of the crosses here reported probably include, in either sex, a larger proportion of tufted than of typical clones.

7. There are indications from the study both of the original tufted clones and of their descendants, that genotypic differences exist, which differences express themselves in the production within individual clones of differing proportions of branches of atypical character.

8. Other probably genotypic differences appearing among the clones here discussed concern the appearance of involucral appendages and the separation or non-separation of spores produced from a single mother

cell. One clone resulting from a cross of tufted × typical presents characters which seem to be constantly different from those of either gametophytic ancestor.

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A COMPARISON OF THE EFFECTS OF X RAYS AND TEMPERATURE ON LINKAGE AND FERTILITY IN DROSOPHILA

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INTRODUCTION

In a series of papers PLOUGH (1917, 1921) has shown that the exposure of females of *Drosophila melanogaster* to temperatures either below or above the usual temperature at which they are reared leads to an increase in their crossover values for certain regions of the second and third chromosomes. The writers of the present article have shown that a similar effect is produced in the second chromosome if the females are exposed to X rays (MAVOR and SVENSON 1923, 1924). In this latter paper attention was drawn to certain differences between the X-ray effect as shown in our experiments and the temperature effect as investigated by PLOUGH. It has seemed of importance to make a careful comparison of the two effects in experiments conducted at the same time and in which the conditions were similar except for the exposure to X rays or the heat treatment. In the experiments to be described the control, X-rayed and heat-treated flies were sisters. The heat treatment was started at the same time as the X-ray treatment. The control and X-rayed females were kept at a uniform temperature of 23°C throughout the experiment and after the heat

treatment which lasted for forty-eight hours, the heat-treated females were kept at the same temperature.

All of the observing and counting of the flies was done by H. K. SVENSON. The statistical treatment and text are the work of J. W. MAVOR. The investigation was made possible through the generous assistance of the Research Laboratory of the GENERAL ELECTRIC COMPANY. We are indebted to Miss L. VAN DER BOGERT of that laboratory for technical assistance.

DESCRIPTION OF EXPERIMENTS

Two experiments are included in this report. The method of the two was the same, the second experiment being a repetition of the first with the exception that some of the X-rayed females received a dose different from those of the first experiment.

Experiment 405

In the first of the experiments to be described, our number 405, two sets of females were used. Those of one set, "A", were from the mating of one wild female with two black purple curved males, made on November 8 at 8:30 p. m., and those of the set "B" were from another similar mating made at the same time. Set A consisted of 22 females which emerged between November 19, 5:10 p.m., and November 20, 11:00 a.m., and set B of 22 females which emerged during the same period. All the females used in the experiment were from pupae isolated in agar test-tubes. There were 12 females (6 from set A and 6 from set B) in the control matings; 20 females (10 from set A and 10 from set B) were X-rayed and 12 (6 from set A and 6 from set B) were exposed to a temperature of 30°C for 48 hours. The heat-treated females were placed in the incubator at approximately the same time, November 20, 7:00 p.m., at which the X-ray treatment was given to the X-rayed flies. The heat treatment lasted for 48 hours, after which the heat-treated females were placed in the same incubator as the X-rayed and control females at the temperature of 23°C. The X-ray dose was that represented by $\frac{2.5 \text{ M A} \times 20 \text{ min}}{(12.6)^2}$, which, according to our method of recording dosage, is represented by 32D, which is a slightly larger dose than was given to the X-rayed flies in experiment 103 reported in our previous paper (MAVOR and SVENSON 1924), where the X-ray dose expressed in this way was 29.4D. The control and treated females remained in each of the first and second bottles for two days, in each of the third, fourth, fifth, sixth and seventh bottles for one day (24 hours),

and in each of the eighth and ninth bottles for two days. The one-day bottles were used in order to determine as accurately as possible the time of incidence of the X-ray and temperature effects. The F_1 coming out in bottles were counted up to and including the seventeenth day after the control and treated females were placed in the bottles.

In table 1 is given the crossover values, sex ratio and fertility for each set of bottles for the control, X-rayed and heat-treated females. The

TABLE 1

Experiment 405. Crossing over, sex ratio and fertility.

BOTTLE	NUMBER OF DAYS AFTER TREATMENT	TREATMENT	NUMBER OF F_1		PERCENT CROSSING OVER		SEX RATIO	FERTILITY
			♀	♂	I	II		
1	1-2	Control	522	451	5.34	20.96	86.4	40.6
		X-rayed	225	230	8.79	21.54	102.2	11.4
		30°C	65	53	4.24	27.12	81.5	14.8
2	3-4	Control	422	419	3.09	12.72	99.3	38.3
		X-rayed	56	43	3.03	11.11	81.5	2.5
		30°C	277	287	2.66	16.49	103.6	28.2
3	5	Control	382	414	3.89	15.70	108.4	72.5
		X-rayed	199	201	3.50	18.25	101.0	23.5
		30°C	248	285	3.00	15.38	114.9	53.3
4	6	Control	382	435	3.92	13.59	113.9	74.2
		X-rayed	87	84	4.68	18.13	96.6	13.1
		30°C	320	346	9.16	16.52	108.1	66.6
5	7	Control	415	408	3.65	17.38	98.3	73.8
		X-rayed	86	94	16.67	30.56	109.3	15.0
		30°C	349	351	14.43	22.14	100.6	70.0
6	8	Control	464	446	2.75	12.53	96.1	91.0
		X-rayed	256	227	15.73	25.67	88.7	40.2
		30°C	407	438	6.75	19.76	107.6	84.5
7	9	Control	401	436	3.35	13.98	108.7	83.7
		X-rayed	496	515	12.17	20.67	103.8	84.2
		30°C	331	342	4.31	19.02	103.3	67.3
8	10-11	Control	342	364	3.82	16.15	106.4	35.3
		X-rayed	394	389	7.02	18.14	98.7	35.6
		30°C	308	317	2.72	14.72	102.9	31.3
9	12-13	Control	459	530	4.55	13.75	115.5	49.5
		X-rayed	485	454	8.20	18.00	93.6	42.7
		30°C	403	366	4.29	16.77	90.8	38.5

crossover value I is for the black-to-purple region, and the value II is for the purple-to-curved region. The sex ratio, as usual, is the number of males per hundred females. The "fertility" is the number of F_1 produced per female per day of egg laying. In the cases in which one female remained for two days in one bottle the fertility is half of the total F_1 (17-day counts) produced in that bottle. The values recorded in this table are represented in the graphs, figures 1 to 3.

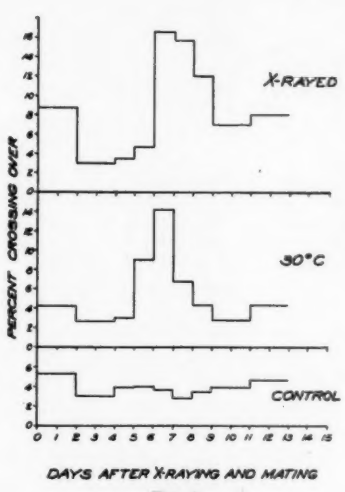

FIG. 1

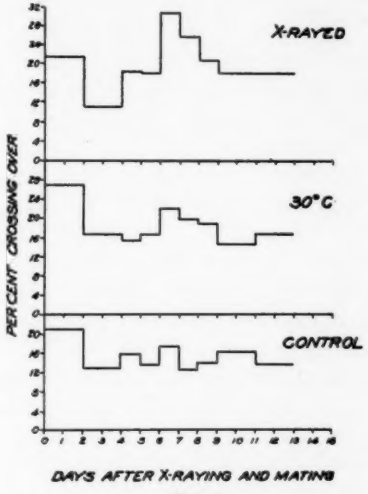

FIG. 2

FIGURES 1 and 2.—Experiment 405. The ordinates indicate percent of crossing over, abscissae the days after X-raying and mating, on which the eggs were laid. Figure 1 is for the black-to-purple region and figure 2 for the purple-to-curved region.

Let us now compare the crossover values for the first region investigated, that between black and purple, shown graphically in figure 1. In the case of the control females the crossover values for this region are quite uniform, starting with a higher value and gradually decreasing with age, a condition already recorded by BRIDGES (1915), PLOUGH (1917) and MAVOR and SVENSON (1924). There is a possibly significant increase during the eleventh to thirteenth days as found by PLOUGH (1917, pp. 177-180). In the case of the heat-treated females, until the end of the fifth day the crossover values are approximately the same as those of the control. There is a marked increase on the sixth day (fourth bottles). The crossover value is greatest on the seventh day (fifth bottles) when it reaches 14.43, the

control for the same bottles being 3.65. An increased crossover value is shown also on the eighth day (sixth bottles), the crossover value here being 6.75 as against 2.75 in the control. It is, however, clear that the increased crossover value occurs only on the fifth, sixth and seventh day after the beginning of the heat treatment. It would further seem likely that the actual duration of the effect of the heat treatment, as expressed in the increased crossover value, is limited to two days, beginning about the middle of the sixth day and lasting until the middle of the eighth day, in which case the duration of the increased crossover value would correspond to the duration of the heat treatment, which was for two days. The findings of this experiment, so far as the heat treatment is concerned, are therefore in complete agreement with those of PLOUGH (1917).

The crossover values of the X-rayed females for region I on the first two days (first bottles) is rather higher than that of the control females. During the third to sixth days (second to fourth bottles) the crossover value is approximately that of the control. On the seventh day (fifth bottles) there is a marked increase in the crossover value to 16.67. The number of F_1 hatched in these bottles, however, was small, 180. On the eighth day (sixth bottles) the crossover value is approximately the same as in the previous bottles. On the ninth day (seventh bottles) there is also a marked increase in the crossover value and there is probably also a significant increase during the tenth to thirteenth days (eighth and ninth bottles). It is to be noticed that the X-ray effect on the crossover value appears somewhat later, one or possibly one-half day later than the effect of temperature and that the X-ray effect persists longer than the effect of temperature.

The sex ratio was investigated, since the work of one of us on the production of non-disjunction by X rays (MAVOR 1921a, b, 1922, 1923, 1924) had indicated that possibly the sex chromosomes were particularly sensitive to X rays. If the sex chromosomes were particularly sensitive to X rays it might be expected that the males which received their single X chromosome from an X-rayed female would be less viable than their sisters which received only one of their two X chromosomes from an X-rayed parent. The data of this experiment shows no significant difference between the sex ratios of the control and treated females.

Let us turn now to a consideration of the crossover values for region II, purple-to-curved. These values are shown graphically in figure 2. The control, although somewhat more irregular, shows the same general condition found in region I, starting with a higher value and decreasing. During the first six days the crossover values of the heat-treated and X-

rayed females are approximately the same as those of the control females. In the case of the heat-treated females, increased crossover values are found on the seventh, eighth and ninth days (fifth, sixth and seventh bottles). After the ninth day the crossover values of the heat-treated females are approximately the same as those of the control females until the end of the experiment. In the case of the X-rayed females an increased crossover value is found on the seventh day (fifth bottles) and on all subsequent days until the end of the experiment, although the greatest increase is found on the seventh, eighth and ninth days.

It is probable that the fertility (number of F_1 per female per day in bottle) determined from one-day bottles is not comparable to the fertility determined from two-day bottles. However, the fertility (figure 3) can be compared in the corresponding bottles of the control, heat-treated and X-rayed, since in each case the females remained for the same number of days, one or two, in the corresponding bottles. In the case of the control females the fertility during the fifth to ninth days was approximately the same on each day as determined from one-day bottles, varying from 72.5 to 91.0 F_1 per female per day. In the case of the heat-treated females the fertility during the first to fourth days was noticeably lower than that of the control females. It gradually increased during the fifth, sixth, seventh and eighth days, reaching the values of the control females on the seventh and eighth days. In the case of the X-rayed females somewhat the same condition is apparent, although the difference between the fertility of these females and those of the control is greater; recovery to the normal fertility is more gradual and is not completed until the ninth day.

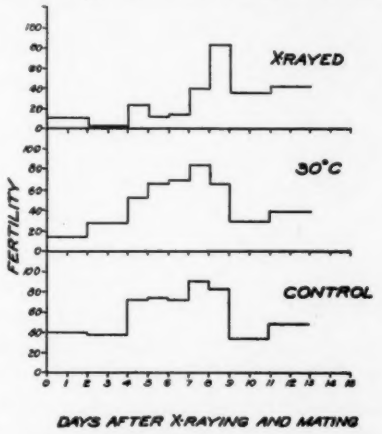

FIGURE 3.—Experiment 405. The ordinates indicate "fertility," i.e., number of F_1 per female per day of egg-laying.

In table 2 is given the counts in the separate crossover classes from which the crossover values given in table 1 were calculated. This table, which we have called a comparative-viability table, is given to show that the increased crossover value cannot be accounted for by any difference in the viability of the different crossover classes.

TABLE 2

Experiment 405. Comparative viability.

BOTTLE	TREATMENT	NON-CROSSOVERS		1		2		1-2	
		+++	−−−	+−−	−++	−−+	++−	−+−	+−+
1	Control	373	354	16	26	81	113	3	7
	X-rayed	159	164	20	14	40	52	3	3
	30°C	47	36	1	2	10	20	1	1
2	Control	372	340	10	12	49	54	2	2
	X-rayed	46	39	2	1	4	7	0	0
	30°C	245	211	9	5	40	53	0	1
3	Control	365	280	10	16	56	64	3	2
	X-rayed	171	144	9	3	28	43	1	1
	30°C	250	186	10	5	34	47	0	1
4	Control	371	305	17	13	51	58	1	1
	X-rayed	65	67	5	3	14	17	0	0
	30°C	257	251	29	19	40	57	7	6
5	Control	334	320	18	8	62	77	2	2
	X-rayed	51	52	10	12	23	24	6	2
	30°C	236	232	48	29	60	71	14	10
6	Control	408	368	7	13	53	56	2	3
	X-rayed	152	144	34	29	51	60	4	9
	30°C	332	296	20	28	80	80	6	3
7	Control	375	321	10	14	51	62	3	1
	X-rayed	385	310	45	62	86	107	8	8
	30°C	282	240	6	17	65	57	1	5
8	Control	322	246	15	9	48	63	2	1
	X-rayed	304	289	21	27	61	74	2	5
	30°C	279	242	5	7	37	50	3	2
9	Control	408	405	19	21	65	66	2	3
	X-rayed	371	337	31	31	67	87	11	4
	30°C	332	281	11	16	58	65	4	2
All bottles	Control	3328	2939	122	132	516	613	20	22
	X-rayed	1704	1546	177	182	374	471	35	32
	30°C	2260	1975	139	128	424	500	36	31

Experiment 406

In the second experiment, our number 406, as in the one previously described, two sets of females were used. Those of set "A" were from the

mating of one wild female with two black purple curved males on January 8 at 2:30 p.m., and those of set "B" were from another similar mating made at the same time. For set A, 32 females were taken which emerged between 9:30 a.m., January 18, and 11:00 a.m., January 19; and for set B, 25 females which emerged between 4:45 p.m., January 18, and 11:00 a.m., January 19. All the females were from pupae isolated in agar test-tubes. In the control matings there were 14 females, 8 from set A and 6 from set B; 15 females, 8 from set A and 7 from set B, were X-rayed with 16D, $\frac{(2.5\,\text{MA} \times 10\ \text{min})}{(12.6)^2}$; 14 females, 8 from set A and 6 from set B, were X-rayed with 32D, $\frac{(2.5\ \text{MA} \times 20\ \text{min})}{(12.6)^2}$; and 14 females, 8 from set A and 6 from set B, were exposed to a temperature of 30°C for two days (48 hours). The heat-treated females were placed in the incubator at approximately the same time, 3:30 p.m., January 19, as the X-ray treatment was started. All of the females were mated, each to two black purple curved males, at approximately the same time. The control pairs and the pairs containing the heat-treated and X-rayed females remained in each of the first and second bottles for two days (48 hours), in each of the third, fourth, fifth, sixth and seventh for one day (24 hours) and in each of the eighth, ninth and tenth bottles for two days, with the exceptions that the matings of set B of the control (six bottles) were not changed into second bottles at the end of the second day, but remained in the same bottles for four days, and that likewise the changing of matings of the control of set B into new bottles at the end of the fifth day was not made, so that they remained in the bottles for two days instead of one day. The values in table 2 have been adjusted for this by including one-half of the F_1 produced by the control of set B in the first bottles (four days) under "first bottles" and one-half under "second bottles" in the table, and similarly one-half of the F_1 produced by the control of set B in the second bottles (two days) under "third bottles" and one-half under "fourth bottles" in the table. The F_1 coming out in the bottles were counted up to and including the seventeenth day after the control or treated females were mated in the bottles. In table 3 are given the crossover values, sex ratio and fertility for each set of bottles for the control, X-rayed and heat-treated females of experiment 406. The values recorded here are shown graphically in figures 4 to 6.

TABLE 3

Experiment 406. Crossing over, sex ratio, and fertility.

BOTTLE	NUMBER OF DAYS AFTER TREATMENT	TREATMENT	NUMBER OF		PERCENT CROSSING OVER		SEX RATIO	FERTILITY
			♀	♂	I	II		
1	1-2	Control	379	405	6.50	19.9	106.9	28.0
		16D	342	313	7.33	21.1	91.5	21.8
		32D	150	142	7.19	25.0	94.7	10.4
		30°C	413	406	5.98	18.7	98.3	29.3
2	3-4	Control	385	429	5.29	16.4	111.4	29.1
		16D	265	284	5.63	16.6	107.2	18.3
		32D	6	3		22.2	50.0	0.3
		30°C	446	466	4.65	13.4	95.7	35.1
3	5	Control	459	428	3.83	15.1	93.4	68.2
		16D	336	324	4.24	17.6	96.4	45.2
		32D	75	73	2.02	20.2	97.3	1.1
		30°C	372	340	6.33	17.0	91.4	59.3
4	6	Control	400	418	5.41	18.4	104.5	62.9
		16D	351	345	5.03	19.7	98.3	49.7
		32D	102	91	7.78	16.1	89.2	19.3
		30°C	372	377	12.0	15.7	101.3	62.4
5	7	Control	554	530	4.52	14.6	95.7	83.4
		16D	382	376	9.10	23.4	98.4	63.2
		32D	135	133	8.99	23.4	98.5	26.8
		30°C	343	369	12.2	21.1	107.6	64.7
6	8	Control	608	613	4.43	14.2	100.8	93.9
		16D	370	371	11.47	23.1	100.3	61.8
		32D	155	138	17.4	23.6	89.0	29.3
		30°C	280	272	11.21	25.2	97.1	50.2
7	9	Control	512	514	3.43	12.3	100.4	78.9
		16D	315	413	10.57	22.9	131.1	60.0
		32D	218	195	13.12	22.3	89.4	41.3
		30°C	294	246	6.30	21.2	83.7	49.1
8	10-11	Control	488	491	2.86	13.1	100.6	37.7
		16D	441	453	8.17	19.7	102.7	37.3
		32D	340	339	10.31	20.8	99.7	34.0
		30°C	433	416	4.59	15.8	96.1	38.6
9	12-13	Control	534	499	3.10	11.2	93.4	39.7
		16D	497	508	7.16	16.2	102.2	41.9
		32D	340	330	8.36	19.7	97.1	37.2
		30°C	443	479	3.37	12.5	108.1	46.1

TABLE 3 (continued)

BOTTLE	NUMBER OF DAYS AFTER TREATMENT	TREATMENT	NUMBER OF		PERCENT CROSSING OVER		SEX RATIO	FERTILITY
			♀	♂	I	II		
10	14-15	Control	550	559	2.80	12.3	101.6	42.7
		16D	552	570	7.67	16.9	103.3	46.8
		32D	368	329	6.89	14.8	89.4	43.6
		30°C	513	459	4.33	14.2	89.5	48.6

The crossover values for region I, black-to-purple, show (figure 4), in the bottles of the control matings, a uniform decrease from the first to eleventh day, after which they remain the same until the fifteenth day. The heat-treated females show a crossover value approximately the

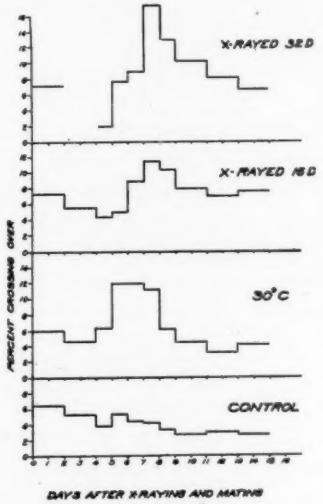

FIG. 4

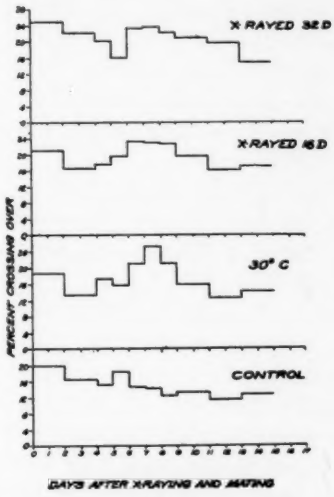

FIG. 5

FIGURES 4 and 5.—Experiment 406. The ordinates indicate percent of crossing over, the abscissae the days after X-raying and mating on which the eggs were laid. Figure 4 is for the black-to-purple and figure 5 for the purple-to-curved region.

same as that of the control females for the first to the fifth days. On the sixth day (fourth bottles) there is a sudden increase in the crossover value from 6.33, the value on the fifth day (third bottles), to 12.00. This high crossover value is maintained for three days (fourth, fifth and sixth bottles), after which time it is approximately that of the

control until the fifteenth day. The females treated with an X-ray dose of 16D show approximately the same crossover value as the control females for the first to sixth days. On the seventh day (fifth bottles) the crossover value is increased to 9.10 and on the eighth day (sixth bottles) to 11.47. In the later bottles the crossover value is not so high, although it remains greater than the control until the end of the experiment, being 7.67 on the fifteenth day as compared with 2.80 in the control bottles. In the case of the females treated with an X-ray dose of 32D the crossover value for the first two days (first bottles) is approximately the same as that in the corresponding control bottles. So few (9) F_1 were produced during the third and fourth days (second bottles) that the crossover value determined from them is without significance. The crossover value on the fifth day (third bottles) is rather low, 2.02, being determined, however, from a rather small number (148) of F_1. The crossover value on the sixth day (fourth bottles) is considerably higher than the control, being 7.78 as compared with 5.41 in the control, although here again it is determined from a rather small number (193) of F_1. On the seventh day the crossover value is slightly higher than that of the sixth day. On the eighth day (sixth bottles) the crossover value reaches a maximum, being 17.4 as compared with 4.43 in the corresponding bottles of the control. After the eighth day the crossover value gradually decreases in the bottles of the succeeding days, but is always higher than in the control bottles, being on the fourteenth and fifteenth days 6.89 as compared with 2.80 of the corresponding bottles of the control matings.

Let us turn now to the crossover values of region II, purple-to-curved (figure 5). The crossover values of the control females show the same gradual decrease with age seen in the previous experiment and in the crossover values of region I in this experiment. The crossover values of the heat-treated females are approximately the same as those of the control females from the first to the sixth day. On the seventh, eighth and ninth days their crossover values are noticeably higher than those of the control females on the same days. After the ninth day the crossover values of the heat-treated females are not significantly higher than those of the control females in the same bottles. In the case of the females X-rayed with 16D the crossover values for the first to sixth days are approximately the same as the crossover values for the control females on the corresponding days. Their crossover values for the seventh, eighth and ninth days are, however, noticeably greater than the corresponding values for the control females, and their crossover values on all succeeding

days until the end of the experiment were greater than the corresponding values of the control females. In the case of the females X-rayed with 32D the crossover values for the first five days were rather high as compared with those of the control, decreasing from 25.0 for the first two days to 20.2 for the fifth day. On the sixth day, however, the crossover value, 16.1, was somewhat lower than the corresponding value for the control, 18.4. The seventh and subsequent days show the usual increase in the crossover value found in X-rayed females. This increase is a maximum on the ninth day, gradually decreases on the subsequent days, but remains greater than that of the controls until the end of the experiment, being on the fifteenth day 14.8 as compared with 12.3, the corresponding value of the control females.

TABLE 4

Experiment 406. Comparative viability.

BOTTLE	TREATMENT	NON-CROSSOVERS		1		2		1-2	
		+++	−−−	+−−	−++	−−+	++−	−+−	+−+
1	Control	405	349	32	26	87	109	6	2
	16D	219	258	21	19	53	77	6	2
	32D	102	101	4	12	26	42	3	2
	30°C	340	287	12	27	75	68	6	4
2	Control	239	236	11	11	36	43	1	5
	16D	215	215	8	20	27	61	3	0
	32D	3	4	0	0	2	0	0	0
	30°C	368	372	18	18	54	71	8	3
3	Control	512	488	23	14	71	108	6	3
	16D	246	279	10	9	52	55	7	2
	32D	64	52	1	1	16	13	1	0
	30°C	294	262	16	19	59	52	5	5
4	Control	175	186	7	15	38	51	4	4
	16D	276	259	11	13	68	58	7	4
	32D	70	81	4	7	16	11	4	0
	30°C	287	272	33	38	42	67	6	4
5	Control	437	448	19	22	61	89	5	3
	16D	268	262	27	24	81	78	13	5
	32D	84	110	10	9	20	29	3	3
	30°C	262	237	27	36	58	68	8	16
6	Control	540	467	17	24	71	89	4	9
	16D	256	249	3	32	68	83	11	9
	32D	96	87	15	26	24	35	5	5
	30°C	171	196	20	26	58	65	10	6

TABLE 4 (continued)

BOTTLE	TREATMENT	NON-CROSSOVERS		1		2		1-2	
		+++	−−−	+−−	−++	−−+	++−	−+−	+−+
7	Control	439	436	5	20	68	48	7	3
	16D	265	242	34	20	74	70	16	7
	32D	134	145	26	16	36	44	6	6
	30°C	201	194	14	17	59	52	1	2
8	Control	428	399	7	17	56	68	3	1
	16D	346	309	25	38	82	84	4	6
	32D	245	237	24	32	65	62	3	11
	30°C	370	315	16	14	61	64	6	3
9	Control	451	436	12	18	51	63	1	1
	16D	405	379	34	24	83	66	6	8
	32D	247	246	23	22	56	65	5	6
	30°C	406	373	9	19	47	65	3	0
10	Control	481	464	6	22	56	77	3	0
	16D	461	403	32	37	71	101	11	6
	32D	269	285	18	22	45	50	7	1
	30°C	417	383	14	20	69	61	3	5
All bottles	Control	4107	3909	139	189	595	745	40	31
	16D	2957	2855	235	236	659	733	84	49
	32D	1314	1348	125	147	306	351	37	34
	30°C	3116	2891	179	234	582	633	56	48

In the case of the sex ratio, as in the previous experiment, no significant change occurs.

A comparison of the fertility of the treated and control females may be made from the graphs in figure 6. These graphs agree fairly well with those for the previous experiment, figure 3. In the case of the control females the fertility was approximately the same as in the corresponding bottles of the previous experiment. In the case of the heat-treated females, it is to be noticed that the fertility on the first to fourth days was somewhat greater than that in the previous experiment and more nearly corresponds to that in the corresponding bottles of the control. This is probably due to the heat treatment having started before the females were mated in this experiment, since PLOUGH (1917, 1921, 1924) has already observed that greater fertility is obtained if this is done than if the flies are already mated when the heat treatment starts, as was the case in our experiment 405. The gradual rise in the fertility of the heat-

treated females on the fifth to ninth days, seen in experiment 405, is not so noticeable here. The X-rayed females show a relatively low fertility during the first to fourth days, that of the females given 32D being much lower than that of those given 16D. In the case of the females given 16D, there is a gradual increase in fertility from the fifth to seventh days, the fertility being always, however, less than that of the control. The fertility is approximately the same on the seventh, eighth and ninth days. In the case of the females given 32D, there is a gradual increase in the fertility from the fifth to ninth days and the fertility of these flies was always less than that of those given 16D. A conspicuous feature of the graphs for fertility is that, as in the case of experiment 405, but shown here more clearly, the fertility of the control, heat-treated and X-rayed females after the ninth day is approximately the same. In other words the effect on fertility, of exposure at the time of mating, to heat or to an X-ray dose which causes almost complete sterility (9 flies were produced by the 14 X-rayed females) on the third and fourth days after mating, disappears on the tenth day after treatment in the case of those flies which continue to reproduce (in this case 10 out of the 14 X-rayed).

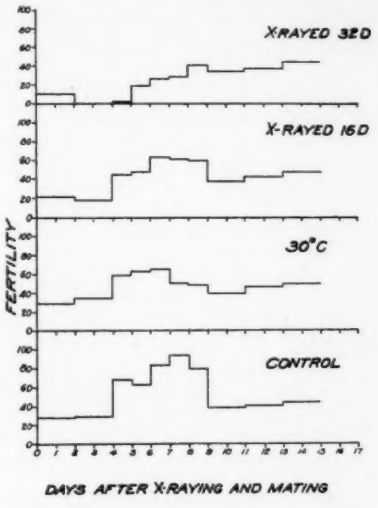

FIGURE 6.—Experiment 406. The ordinates indicate "fertility," i.e., number of F_1 per female per day of egg-laying.

In table 4 is given the counts of the separate crossover classes. In this table counts of the first bottles of set B, control (first to fourth days) are all included under "first bottles" in the table, and the counts of the second bottles (fifth and sixth days) are all included under "third bottles" in the table. Since the table is given primarily to show that there is no great difference in the viability of the complementary classes it seemed inadvisable to make any adjustment in it.

It will have been evident from the above description that the second of our experiments, number 406, confirms the first, number 405. For statis-

tical treatment these two experiments have been considered together in the next section.

A number of F_1 flies from the fifth bottles (seventh day) of the control and X-rayed females were bred to determine whether the increased crossover value was transmitted to them. The data obtained from these matings have already been reported (MAVOR and SVENSON 1924 b). The conclusion to be drawn from these is that there is no indication of the increased crossover value being transmitted.

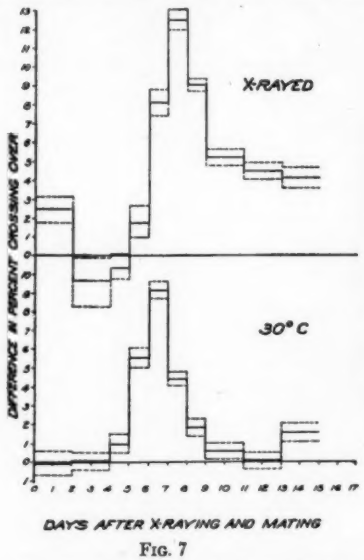

FIG. 7

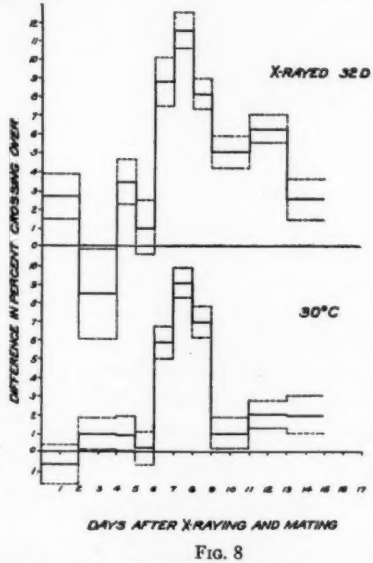

FIG. 8

FIGURES 7 and 8.—Combined results of experiments 405 and 406. The ordinates indicate the difference between the percent of crossing over in the treated and control females, the abscissae the days after the beginning of the treatment when the eggs were laid. The continuous lines give the actual values obtained for the differences, and the interrupted lines the differences plus or minus the probable errors. Figure 7 represents the black-to-purple region, and figure 8 the purple-to-curved region.

Combined results of the two experiments

In table 5 are given the crossover values, sex ratios and fertilities obtained by adding together the counts obtained in the two experiments. The quantities in this table therefore represent the weighted averages of these quantities in the two experiments. The probable error of the difference between the crossover value of the treated and control females has been determined in each case. The data on crossing over, in this table, are represented graphically in figures 7 and 8. Here the continuous line

TABLE 5

Experiments 405 and 406. Crossing over, sex ratio and fertility.

BOTTLE	NUMBER OF DAYS AFTER TREATMENT	TREATMENT	NUMBER OF		PERCENT CROSSING OVER		DIFFERENCE P.E.-Difference		SEX RATIO	FERTILITY
			♀	♂	I	II	I	II		
1	2	Control	901	856	5.86	20.5			95.0	33.8
		X-rayed	375	372	8.30	23.1	3.53	2.25	99.2	11.0
		30°C	478	459	5.76	19.7	.15	.67	96.0	26.0
2	2	Control	807	848	4.17	14.6			105.1	33.1
		X-rayed	62	46	2.78	12.0	.92	.83	74.2	1.6
		30°C	723	753	4.20	15.6	.06	1.11	104.1	32.1
3	1	Control	841	842	3.92	15.4			100.1	70.1
		X-rayed	274	274	3.28	18.8	.99	2.86	100.0	17.7
		30°C	620	625	4.90	16.3	2.01	1.02	100.8	56.6
4	1	Control	782	853	4.53	16.0			109.1	68.1
		X-rayed	189	175	6.32	17.9	2.20	.70	92.6	15.8
		30°C	692	723	10.04	16.2	9.69	1.04	104.5	64.3
5	1	Control	969	938	4.20	15.8			96.88	79.5
		X-rayed	221	227	12.28	24.6	11.38	6.80	102.7	20.4
		30°C	692	720	13.31	21.6	19.22	6.75	104.0	67.2
6	1	Control	1072	1059	3.94	13.3			98.8	92.7
		X-rayed	411	365	16.49	24.7	22.82	12.01	88.8	35.3
		30°C	687	710	8.30	22.3	12.15	11.74	103.3	66.5
7	1	Control	913	950	3.38	13.0			104.1	81.0
		X-rayed	714	710	12.43	21.1	26.56	10.13	99.4	64.7
		30°C	625	588	5.19	20.0	4.03	8.24	94.1	57.8
8	2	Control	830	855	3.26	14.4			103.0	36.6
		X-rayed	734	728	8.55	19.4	12.02	5.91	99.2	34.8
		30°C	741	733	3.80	15.3	1.25	1.15	98.9	35.1
9	2	Control	993	1029	3.81	12.5			103.6	44.0
		X-rayed	825	784	8.27	18.7	10.34	8.39	95.0	40.2
		30°C	846	845	3.78	14.4	.05	2.68	99.9	42.3
10	2	Control	550	559	2.80	12.3			101.6	42.7
		X-rayed	368	329	6.89	14.8	8.25	2.35	89.4	43.6
		30°C	513	459	4.32	14.2	3.16	1.99	89.5	48.6

represents the difference in percent between the crossover value of the treated and control females and the dotted lines on either side of the continuous line represent the probable error of the difference.

Considering first the black-to-purple region (figure 7), and comparing the crossover values of the heat-treated and control females, it is seen that there is no significant difference between them during the first five days. On the sixth, seventh and eighth days the differences are certainly significant, the difference being greatest on the seventh day. On the ninth day the difference is possibly significant, being 4.03 times the probable error of the difference. After the ninth day the differences are clearly not significant. Comparing now the X-rayed (only those given a dose of 32D are included in this table) and control females it is seen that here the differences during the first six days are not significant. The first significant difference appears on the seventh day and the greatest difference on the eighth day. The differences on all subsequent days until the end of the experiment are significant, the differences during the twelfth and thirteenth days being 10.34 times the probable error of the difference and during the fourteenth and fifteenth (experiment 406 only) 8.25 times the probable error of the difference.

In the case of the purple-to-curved region (figure 8) a significant difference in the crossover value appears simultaneously in the heat-treated and X-rayed females during the seventh day. In both the heat-treated and X-rayed females the difference reaches a maximum during the eighth day, being during the ninth day approximately the same as during the seventh day. On the subsequent days the heat-treated and X-rayed females behave differently. While the heat-treated females show no significant difference from the control, the X-rayed females show significant differences during the tenth and eleventh, and twelfth and thirteenth days. The X-rayed females do not, however, show a significant difference during the fourteenth and fifteenth days.

The main points with regard to crossing over, brought out by the experiments, will now be summarized. A comparison of the effect of temperature and X rays on crossing over in the first region investigated, black-to-purple, showed that the first significant increase appeared in the heat-treated females during the sixth day after the commencement of the heat treatment and in the X-rayed females during the seventh day after X-raying. The maximum effects were found on the seventh and eighth days, respectively, and an X-ray dose of 32D, $\frac{(2.5\ \text{MA}\times 20\ \text{min})}{(12.6\ \text{cm})^2}$, had a greater effect than exposure to a temperature of 30°C. While in the case of the

heat-treated females the increase in the crossover value lasted for three days only and is possibly to be considered as involving only two days, in the case of the X-rayed females it lasted until the end of the experiment, i.e., for nine days.

In the case of the second region investigated, purple-to-curved, the results were somewhat different. The first significant increases in crossing over in the heat-treated and X-rayed females appeared simultaneously on the seventh day. In the heat-treated females the purple-to-curved and black-to-purple regions behaved differently, the increase in the crossover value of the latter region appearing one day earlier than in the case of the purple-to-curved region. The greater increase in the crossover value of the purple-to-curved region lasted, however, for three days, i.e., as long as in the case of the black-to-purple region and the graph for the increased crossing over has the same form in the two regions. A significant increase in the crossover value of the purple-to-curved region in the heat-treated females occurred during the ninth day, one day later than the last significant increase occurred in the black-to-purple region. While in the heat-treated females no significant increase in the crossover value occurred after the ninth day, in the X-rayed females significant increases were found up to the thirteenth day.

DISCUSSION

Comparison of the times at which the effects appear

The primary object of this investigation was to repeat our earlier experiments under conditions which would make possible a direct comparison of the effects of X-ray and heat treatment on crossing over. In our two earlier experiments already reported (MAVOR and SVENSON 1924a) the effects of the X rays on crossing over were noticed rather sooner after the treatment than was to be expected from the work of PLOUGH (1917) on the effect of temperature on crossing over. This alone suggested the advisibility of a repetition of the experiments. In the experiments which we are now reporting the X-ray effect becomes evident at approximately the same time as the temperature effect, when the treatments are started at the same time. We are unable completely to explain the earlier incidence of the X-ray effect in our first two experiments. Certain points may, however, be noted. In these experiments (MAVOR and SVENSON 1924 a), the control and X-rayed females were left in the bottles for three days. In the first experiment, 103, the X-ray treatment was given to the females on the day before they were mated

so that the second bottles really contained eggs laid on the fourth to seventh days and probably mostly eggs laid on the seventh day after X-raying, since females are relatively sterile for the first six days after X-raying. The second experiment, 404, does not admit of a similar explanation. However, the differences divided by the probable errors for the second bottles were not large, being in the case of the black-to-purple region 6.6, and the purple-to-curved region 4.6.

In the experiments now reported, the increased crossover value for the black-to-purple region was found in the case of the heat-treated females in eggs laid approximately one day before the eggs which showed the increase in the case of the X-rayed females. The question thus arises whether there is any real difference in the time at which the crossover value appears in the two cases. In the absence of exact knowledge as to the effects which X-ray and heat treatments have on the rate of oögenesis it would not seem of value at present to carry the comparison further.

In the case of the purple-to-curved region the increased crossover value appeared simultaneously in the X-rayed and heat-treated females. It is to be noticed, however, that there is a difference in the time at which the increased crossover value appears in the black-to-purple and the purple-to-curved region in the same heat-treated females. It is not possible to compare our data directly with those of PLOUGH (1917) on this point, since he used two-day where we used one-day bottles.

Comparison of the duration of the effects

Both of the experiments show that when females are treated with X rays immediately before mating, their crossover value is increased in eggs laid from the seventh to the fifteenth day after the treatment, and confirm the results of our earlier experiments that exposure to X rays for a comparatively short time, in this case 20 minutes, leads to an increased crossover value over a long period. It may be asked whether this prolongation of X-ray effect is not really due to a retardation in the process of oögenesis due to the X-ray treatment. It would seem difficult to give a conclusive answer to this question. It may be observed, however, that during the latter days of the experiment, during which the crossover values for the X-rayed females were high, the fertility of these females was approximately the same as that of the control and heat-treated females, and that there was no evidence of retardation of development in the hatching of the F_1 from these females. Any retardation in the process of

oögenesis would seem likely to cause either a retardation in the development of the offspring or an alteration in the fertility, or both.

Comparison of the effects of X rays and radium

In a recent paper PLOUGH (1924) has reported some experiments with radium radiations. He exposed the flies in a glass tube in contact with a glass tube containing 50 mg of pure radium bromide. After a 40-minute exposure he found that the crossover value for the black-to-purple region was increased on the 9th to 12th days to 9.50 as compared with 3.60 for the control. The difference in this case, 5.9 percent, divided by the probable error of the difference being 4.18. He states further that exposure for one hour to the radium "had a very marked lethal effect on the eggs." The number of flies which hatched, "less than 100," being too small to give significant crossover values. The increase in crossing over after radium treatment observed by PLOUGH is considerably less than the increase observed by us, even after the weaker of the X-ray doses. PLOUGH's data are, however, rather too meagre, as he admits, to lead to definite conclusions. In his radium treatment PLOUGH exposed his flies to both the beta and gamma rays. Only the gamma rays are strictly comparable to X-rays. It may, therefore, have been that the increased crossing over was due to the gamma rays only, there being an additional lethal effect due to the beta rays. This would account for PLOUGH not having observed as great an increase in the crossover value due to radium treatment as we obtained in our X-ray experiments. If this supposition is correct a sufficient exposure of the flies to gamma rays only, ought to cause a greater increase in crossing over comparable to the increase caused by X-ray treatment.

Fertility and crossing over

The fertility was studied in the experiments because it was thought that it might bear some relation to the modification of the crossover value. During the time in which the crossover value increases in the X-rayed females (seventh to ninth days) their fertility is less than that of the control or heat-treated females. There is also during these days a gradual increase in the fertility corresponding to the increase in the crossover value. There is, however, also a gradual increase in the fertility of the control females during this time. After the ninth day the fertility of all the females is approximately the same.

SUMMARY

1. A comparison has been made between the effects of X rays and heat treatment in modifying the crossover value in the black-to-purple and the purple-to-curved regions of the second chromosome.

2. The effects of X rays and temperature in causing an increase in the crossover value appeared at approximately the same time, in the F_1 from eggs laid on the sixth or seventh day after the beginning of the X-ray or heat treatment.

3. In the case of the heat treatment the increase in the crossover value of the black-to-purple region appears one day earlier (eggs laid on the sixth day) than in the purple-to-curved region (eggs laid on the seventh day).

4. The increased crossover value caused by the X-ray treatment persisted until the end of the experiment and was evident in eggs laid on the fourteenth and fifteenth days after a single X-ray treatment lasting twenty minutes.

5. The lethal effect of the X-ray doses on the eggs in the ovary disappeared ten days after the treatment.

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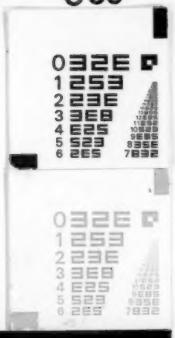
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